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Matured and Young *Anacardium occidentale* Leaves Extract for Amylase Inhibitor: An Alternative to Antidiabetic

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Abstract

Diabetes Mellitus (DM), commonly known as diabetes, is one of the diseases that recorded high mortality rate. World Health Organization (WHO) reported in 2016, about 1.6 million deaths were directly caused by diabetes. Other studies had reported there were about 387 million diabetic patients globally in 2014. While it is expected that 145 million people with diabetes would be in the South East Asia region and 2.5 million people are in Malaysia in 2025. Based on statistics showed by International Diabetes Federation (IDF) the number of diabetic patients will rise to 700 million by 2045. This study reports on finding a new source of amylase inhibitor that contribute to minimize the percentage of people with diabetic disease in Malaysia generally and Selangor specifically. Matured and young leaves of Anacardium occidentale used in the study were extracted using aqueous extraction method. Ten grams of dried leave was dissolved in 100 ml of distilled. The mixture was filtered using Whatman's filter paper No. 1. Active compounds extraction from Anacardium occidentale leaves using aqueous method of different concentrations, different incubation periods and different incubation temperature indicated most of the extracted sample from different types of Anacardium occidentale leaves (young and matured) revealed their effectiveness as antidiabetic. Extract from the matured leaves showed its ability to reduce amylase activity from 0.12 U/ml to 0.06 U/ml when it was incubated for 15 minutes. The amylase activity was recorded at 0.053 when it was further incubated for 30 minutes. Nonetheless, the amylase activity remains unchanged when it was further incubated for 45 and 60 minutes. On the other hand, the extract from young leaves revealed there is slight reducing in the amylase activity (0.11 U/ml) for 15 minutes incubation. However, the extract showed its ability to reduce the amylase activity to 0.107 U/ml, 0.085 U/ml and 0.062 U/ml when it was further incubated for 30, 45 and 60 minutes, respectively. It is anticipated that consuming Anacardium occidentale leaves (Cashew nuts leaves) would enhance the level of healthier community both domestically and globally which is in line with the National Vison Policy 2020 and the 4th National Mission Thrust which both aim at improving the standard and sustainability quality of Malaysians life.

Keywords: *diabetes mellitus, extract, Anacardium occidentale*

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INTRODUCTION

Diabetes Mellitus (DM), commonly known as diabetes, is one of the diseases that recorded high mortality rate. Based on statistic of World Health Organization (WHO) in 2016, an estimated 1.6 million deaths were directly caused by diabetes. Al-Naggar et al., (2017), on the other hand, reported there were about 387 million diabetic patients globally in 2014, whereas a study conducted by Letchuman et al., (2010) indicated the European region has recorded the highest number of people with diabetes with 48 million and Western Pacific region with 43 million. Furthermore, it is expected in 2025 the South East Asia region will have greatest number of people with diabetes that estimated around 145 million people and the number will rise to 700 million by 2045 based on statistics showed by International Diabetes Federation (IDF). In Malaysia, around 2.5 million people were reported to be categorized as diabetic patients (Al-Naggar et al., 2017). Recent statistics showed approximately 463 million adults worldwide, ages between 20-79 years are diagnosed with diabetic disease (International Diabetes Federation).

Anacardium occidentale leaves possess many useful phytochemicals include antioxidants, anti-breast cancer and antiplasmodial and enzymes inhibitors. However, there are not many studies on other parts of the plant including fruits and nuts have been reported so far. Hence, the present study aims to investigate the presence of alpha amylase inhibitor activity in the *A. occidentale* fruits and nuts that can be used as alternative sources of antidiabetic. The study is designed to include various research activities such as preparation of fruits, nuts and leaves (young and old leaves) extracts using different extraction solvents, determination of optimal extraction solvent for extracting the alpha amylase inhibitor and determination of the optimum alpha amylase inhibitor from fruits, nuts and leaves as antidiabetic agent. This study is expected to find a new source of alpha amylase inhibitor that contribute to minimize the percentage of people with diabetic disease in Malaysia generally and Selangor specifically. Besides, these study also to enhance the level of healthier community both domestically and globally through adoption of healthy food culture which is in line with the National Vison Policy 2020 and the 4th National Mission Thrust which both aim at improving the standard and sustainability quality of Malaysians life.

It has been reported that there were about 387 million diabetic patients globally in 2014. Surprisingly, 2.5 million of them were in Malaysia (Al- Naggar et al.,2017). Even though, study conducted by Letchuman et al. (2010) indicated the European region was recorded as the highest number of people with diabetes with 48 million and Western Pacific region with 43 million. However, it is estimated that in 2025, the Diabetes Atlas will change and the greatest number of people with diabetes would be in the South East Asia region with 145 million people. Diabetes Mellitus (DM) or commonly known as diabetes, is a group of metabolic diseases where there are high blood sugar levels over a prolonged period (Prathima et al.,2013) and if the disease were left untreated, it can cause many complications (Parker et al.,2001). There are different types of diabetes complications, firstly acute complications, including diabetic ketoacidosis and non-ketotic hyperosmolar coma. Secondly, serious long-term complications, including kidney failure, cardiovascular disease, stroke, foot ulcers and damage to the eyes (Lynd et al.,2005). According to

diabetes may occur due to either insufficient of insulin produced by the pancreas or the cells of the body did not respond properly to the insulin produced (Jonas et al.,2000).

Anacardium occidentale also knowns as cashew nuts, is categorized under family of Anacardiaceae which is a native to Brazil (Chan et al.,2017). In Malaysia, *A. occidentale* grows especially on coastal area and estimated about 480 hectare of plantation area in 2010 (Animagro, 2015) while this small evergreen plant continuously being planted for their nut production in Indonesia and Vietnam (Norzielawati, 2015). According to Heuze et al. (2016), *A. occidentale* is known as multipurpose plant species, which almost all parts are edible and possess ethnomedicinal properties. For instance, the anacardic acids content from nutshell liquid is effectively used against tooth abscesses. In additions, an antidiarrheal property also been reported while powder from seeds used for antivenom for snake bites (Akash et al.,2009). In Southeast Asian countries, particularly Malaysia, Thailand and Indonesia, the young and tender leaves of *A. occidentale* are regularly consumed as condiment. Many pharmacological properties include antioxidant, antibacterial, antiviral, antifungal, hypoglycaemic, hypolipidemic, anticholesterolemic, anti-ulcerogenic, anti-hypertensive, analgesic and anti-inflammatory activities have been reported from *A. occidentale* (Chan et al.,2017).

A detailed study of the phytochemical and pharmacognostical parameters had been carried out by Yogini et al., (2012) and results of the study revealed the presence of carbohydrates, proteins, saponin glycosides, flavonoids, alkaloids, tannins and phenolic compounds in ethanol and aqueous extract of *A. occidentale* leaves. On the other hand, Chan et al., (2017) reported that flavonoids and phenolic acids are the major metabolites found in the *A. occidentale* leaves. It is considered as a very useful plant that possesses many pharmacological properties. Nonetheless, due to less studies on alpha amylase inhibitor extracted from the plant, hence, this study is very significant to investigate the significant of the *A. occidentale* as a source of alpha amylase inhibitor used as antidiabetic agent.

Phytochemicals are plant metabolites which act as natural defense systems for host plants, and also provide characteristic color, aroma and flavor in specific plant parts. They are a group of non-nutrient compounds that are biologically active when consumed by human. Many phytochemicals are health-promoting and can prevent many diseases. Phytochemical analysis revealed the presence of alkaloids, tannins, anthraquinolones, glycosides, and phenols in both ethanol and aqueous extracts of *A. occidentale* dried fruit. It is also reported the presence of alkaloids; tannins and saponins in *A. occidentale* stem extract.

Several studies have reported rich variety of secondary metabolites in *A. occidentale extracts*. The pharmacological properties of medicinal plants have been attributed to their rich secondary metabolites. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs.

RESEARCH METHOD

Location Study

This study was conducted in the Bioprocess Laboratory, Department of Science and Biotechnology, Faculty of Engineering and Life Sciences, Universiti Selangor (Unisel), Bestari Jaya, Selangor, Malaysia.

Sample Collection

The leaves of *A. occidentale* were collected from rural areas around Kampung Bestari Jaya, Selangor.

Extraction of the Leaves

The leaves undergone were rinsed, dried, cut, ground, soaked with water, filtered, evaporated and freeze-dried prior using for amylase inhibitor process. The extracts were diluted with water and filtered under sterile filtration process to obtain liquid form of the extract. The leaves extract concentration was 20 mg/ml.

Amylase Assay

Quantitative amylase enzyme assay is conducted by incubating 1 ml of the plant extract (sample) in 1 ml of the substrate (2% of starch dissolved in Phosphate Buffer, pH 7). The mixture is inoculated at 37°C for 15 min. The reaction mixture is then stopped by adding 5 ml of DNSA and further incubated at 100°C for 5 min. The mixture is then cooled at room temperature and the absorbance is read at 540nm. The amylase enzyme is assayed using DNS method (Bernfeld, 1955).

RESULTS AND DISCUSSION

Sample Preparation

Fresh young and matured leaves of *A. occidentale* will be collected at Kampung Bestari Jaya, Selangor. The leaves were washed thoroughly under tap water (Figure 1) and were oven-dried at temperature 60°C for three days as shown in Figure 2. The dried leaves were pulverized into uniform powder using a household blender and were sieved to obtain a fine powdered particle. 500 grams of powdered leaves of both matured and young leaves (Figure 3) were macerated in water and then were filtered and concentrated on a rotary evaporator. The dried extracts will be stored at 4°C for further analysis.

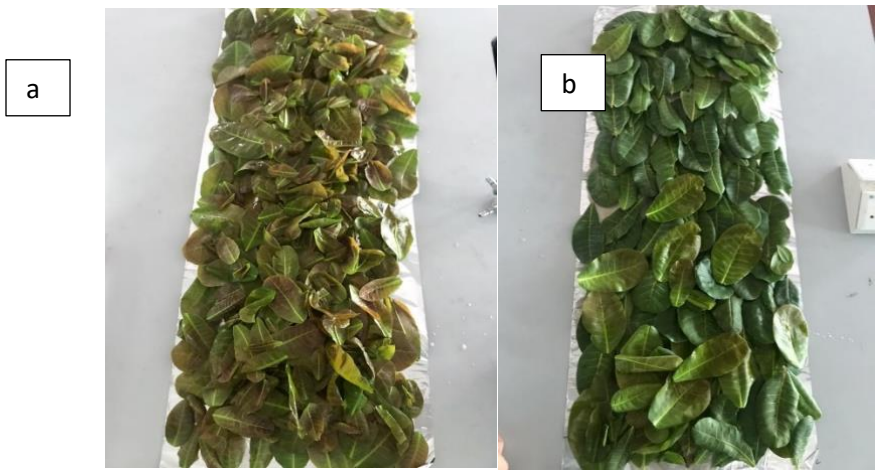

Figure 1: Both young (a) and mature (b) leaves of *Anacardium occidentale*.

Figure 2: Leaves are dried in oven for 3 days at 60 °C

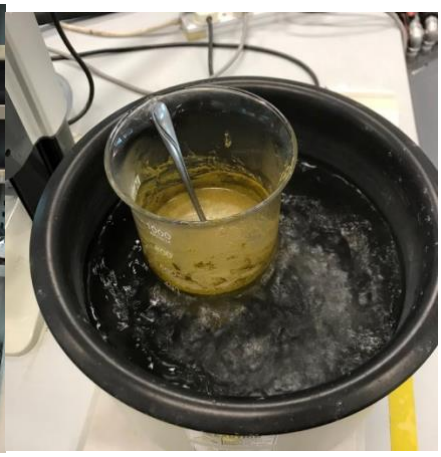

Figure 3: Boiling of powdered leaves dissolved in distilled water for 10 minutes.

Amylase Assay

Amylase enzyme assay without plant extract, which is considered as a control, revealed its activity at 0.12 U/ml. While incubation of amylase enzyme with extract of mature leaves of *A. occidentale* at different periods of time ranging from 15 to 60 minutes prior to enzyme assay has shown reducing in the enzyme activity as indicated in Figure 4. The amylase enzyme activity was found at 0.06 U/ml and 0.053 U/ml when it was incubated for 15 and 30 minutes, respectively. Nonetheless, the amylase enzyme activity remains unchanged when it was further incubated for 45 and 60 minutes. On the other hand, the amylase enzyme assay in the presence of extract from young leaves revealed a slight reducing in the amylase activity (0.11 U/ml) for 15 minutes incubation. However, further incubation for 30, 45 and 60 minutes the extract showed its ability to reduce the amylase activity to 0.107 U/ml, 0.085 U/ml and 0.062 U/ml, respectively (Figure 5).

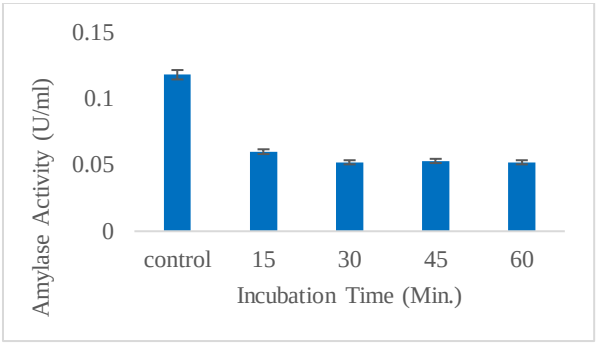

Figure 4: Amylase activity before (control) and after incubation with *A. occidentale* mature leaves extract at different incubation times.

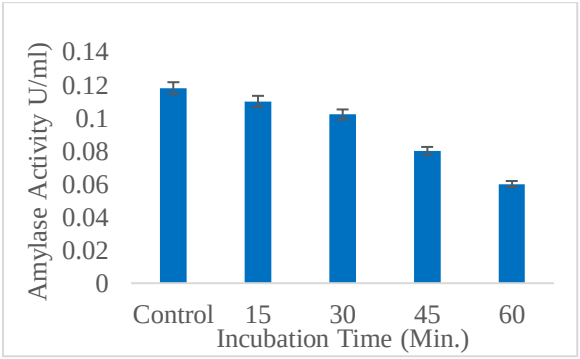

Figure 5: Amylase activity before (control) and after incubation with *A. occidentale* young leaves extract at different incubation times.

Discussion

Effectiveness of both mature and young extracts of *A. occidentale* leaves in this study has been proven. The *A. occidentale* leaves extracted using aqueous method managed to reduce the amylase enzyme activity. Mature leaves have shown to be more effective than young leaves in reducing amylase activity (Figure 6). In addition, the relative enzyme activity indicated the young leaves required more incubation time to manifest their effectiveness compared to the mature leaves (Figure 7).

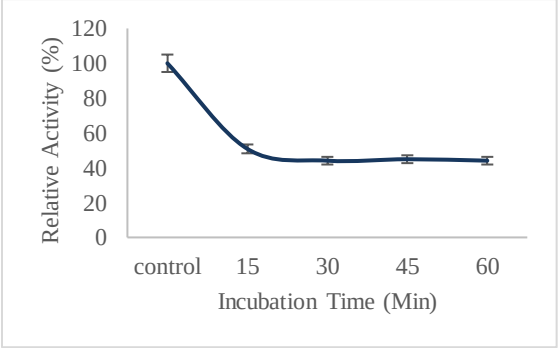

Figure 6: Relative Amylase activity before (control) and after incubation with *A. occidentale* mature leaves extract at different incubation times.

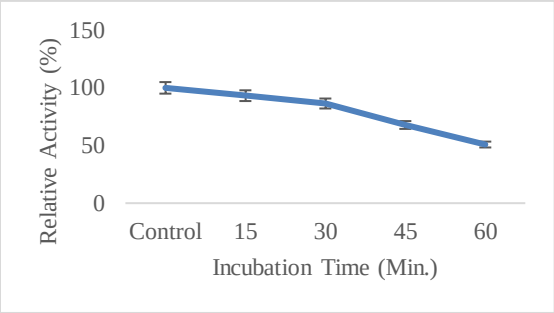

Figure 7: Relative Amylase activity before (control) and after incubation with *A. occidentale* young leaves extract at different incubation times.

Study on the effectiveness of *A. occidentale* leaves extract have been reported by many researchers. For instance, Abu et al., (2020), reported on the aqueous extraction of *A. occidentale* leaves that was able to reduce diabetogenic action on Wistar rats. Besides, Jaiswal et al., (2016) also reported their successful of extracting antidiabetic activity of ethanol extract of *A. occidentale* leaves n-streptozotocin diabetic rat. Recently, a study by Damsuk et al., (2021) showed the antidiabetic potential of A. occidentale shoots and leaf extracts. The studies suggest that A. occidentale potentially be used as a therapeutic agent against diabetes and as a functional food component.

Result obtained in the current study showed some similarity to the study reported by Lalitha and Jannatul Firdhouse, 2016, where the aqueous extract of *Annona reticulata, Amaranthus polygonoides* and *Kedrostis foetidissima* leaves showed their effectiveness as amylase inhibitor that managed to reduce almost 60% of the amylase activity. Nonetheless, the current study indicated better performance as compared to the study reported by Oyedemi et al., (2017) where the α-amylase inhibitor from methanol extract of *A. occidentale* leaves only managed to inhibit about 26.39%.

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REFERENCES

Abu, O. D., Imafidon, K. E. and Obayuwana, O. (2020). Effect of Aqueous Extract of Anacardium occidentale Leaves on Blood Glucose Level and Lipid Profile of Diabetic Rats. Global Scientific Journal, 8 (10) ISSN2320-9186.

Akash, P.D., Vishal, D.J. and Arun, B.J. (2009) Antimicrobial Screening of Different Extract of Anacardium occidentale Linn. Leaves. International Journal of ChemTech Research CODEN (USA), 1(4): 856-858

Anand, G.R., Basaviah, M., Shetty, A.V., (2015). In Vitro Antimicrobial and Cytotoxic Effects of Anacardium occidentale and Mangifera indica in Oral Care. J Pharm BioAllied Science, 7:69-74

Arekemase, M.O., Adams, A. I., Ibrahim, T. O., Babashola, K. D., Ahmed, M. I. and Dunmoye, T. D. 2020. The Efficacy of Extracts of the Leaf and Bark of Cashew Plant (Anacardium occidentale) on Bacterial Isolates Isolated from Banana (Musa paradisica). Nigerian Journal of Microbiology, 33(2): - 4821 – 4828.

Candiotto, F. B., Freitas-Júniorb, A. C. V., Neric, R. C. A., Bezerrac, R. S., Rodriguesd, R. V., Sampaiod, L. A. and Tesser, M. B. (2018). Characterization of digestive enzymes from captive Brazilian flounder Paralichthys orbignyanus, Brazilian Journal of Biology, 78 (2): 281-288.

Chan, W.C., Shigeyuki, B., Chan, H.T., Mami Kainuma, Tomomi Inoue and Wong, S.K. Ulam Herbs: (217). A Review on Medicinal Properties of Anacardium occidentale and Barringtonia racemosa. Journal of Applied Pharmaceutical Science, 7(02): 241-247.

Dumsuk, T., Tedphum, T., Sukkrong, C. and Lila, M. A. (2021). Anti-Diabetic Potential of Cashew Nut (Anacardium occidentale) Shoots and Leaves Extracts under Simulated In Vitro Digestion. Science and Technology Asia, 26(2): 138-144.

Edy Brito, Liana Mendes, Deborah Garruti, Guilherme Zocolo, Marcelo Lima. 2020. Structure-Astringency Relationship of Anacardic Acids from Cashew Apple (Anacardium Occidentale L.). Page 92. http://doi.org/10.26434/chemrxiv.12336704.v1

Ermis, E. (2017). Halal status of enzymes used in food industry. Trends in Food Science & Technology 64, 69-73

Gurung, N., Ray, S., Bose, S. and Rai, V. (2013). A Boarder View: Microbial Enzymes and Their Relevance in Industries, Medicine, and Beyond, BioMed Research International., Article ID 329121

Hassan, I A, Abdulraheem I, Emun H O. and Shonowo O M. (2019). Sensitivity of Cashew (Anacardium occidentale) Leaf extract against selected urinary tract pathogens. Journal of Advances in Medicine and Medical Research 29(6): 1-6, 2019; Article no. JAMMR.46486, ISSN: 2456-8899.

Heuze,V., Tran,G.,Haasoun, P., Bastainelli, D. and Lebas, F. Cashew (Anacardium occidentale L.) (2016). Nuts and By Products. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. http://www.feedipedia.org/node/56. Last updated on October, 26: 16:09

Jaslyn Jie Lin Lee, Xi Cui, Kong Fei Chai, Guili Zhao and Wei Ning Chen. (2020). Interfacial Assembly of a Cashew Nut (Anacardium occidentale) Testa Extract onto a Cellulose-Based Film from Sugarcane Bagasse to Produce an Active Packaging Film with pH-triggered Release Mechanism. Food and Bioprocess Technology. Vol. 13, pg. 501-510.

Lalitha, P and Jannatul Firdhause, M. (2016). Assessment of α-amylase Inhibitory Action of Some Edible Plant Sources. Innovare Journal of Sciences, 4 (3):1-7.

Manasa, M.K., Swamy, H.C.S., Vivek, M.N., Kumar, T.N.R., Kekuda, T.R.P., Antibacteria Efficacy of Pimenta dioica (L.) (2013). Meril and Anacardium occidentale L. Against Drug Resistant Urinary Tract Pathogens. J Appl Pharm Sci, 3:72-74

Mohd Aliff, A. M., Izhar Hafifi, Z. A., Hayati Adilin, M. A. M. and Chemah, T. C. (2015). Issues of halal food implementation in Malaysia. Journal of Applied Environmental and Biological Sciences 5(6): 50-56.

Norzielawati, S. Anacardium occidentale L (2015). Institut Penyelidikan Perhutanan Malaysia (FRIM), 160(9)

Oyedemi, S. O., Oyedemi, B. O., Ijeh, I. I., Ohanyerem, P. E., Goopoosamy, R. M. and Aiyegoro, O.A. (2017). Alpha-Amylase Inhibition and Antioxidative Capacity of Some Antidiabetic Plants Used by the Traditional Healers Southeastern Nigeria. The Scientific World Journal, https://doi.org/10.1155/2017/3592491

Ram Kumar, P., Princess Jeba, D., Alwin David, S. and Chinnasamy, M. (2019). Phytochemical and Antimicrobial Studies of Biosynthesized Silver Nanoparticles Using Anacardium Occidentale Fruit Extract. IJRAR March 2019, Volume 6, Issue 1. E-ISSN 2348-1269, P- ISSN 2349-5138.

Ranjbar, M., Zibaee, A. and Sendi, J. J. (2014). Purification and characterization of digestive lipase in the midgut of Ectomyelois ceratoniae Zeller (Lepidoptera: Pyralidae), Frontier in Life Sciences, 8(1): 64–70.

Sija S.L, Anand Adithya V, Sasankan Arathy and Anil Arun. 2019. In vitro antioxidant activity and antibacterial activity of ethyl acetate extract of medicinal Tree Species, Anacardium occidentale L. and Mangifera indica L. International Journal of griculture, Environment and Biotechnology. Vol. 12 (2). Pg. 115-120. ISSN: 0974-1712. Online ISSN: 2230-732X. Article DOI: 10.30954/0974-1712.2019.6.

Thomas, B.T., Soladoye, M.O., Adegboyega, T.T., Agu, G.C.and Popoola, O.D. (2015). Antibacteria and Anti-Inflammatory Activities of Anacardium occidentale Leavesand Barks Extracts.Nigerial Journal of Basic and Applied Sciences, 23 (1)

Yogini, J., Vinayak N., Pratima T., Satish G., and Ashok V. Pharmacognostic and Preliminary Phytochemical Investigations of Anacardium occidentale (Linn.) Leaves. Int J Pharm Sci., 4(3): 625-631