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Research paper



In-Vitro α-Glucosidase Inhibitory Activities of Muntingia Calabura Linn.

N.I.I. Nor Azman[1], N. Hashim[2], R. Ahmad[3]

[1]*School of Chemistry & Environment, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, 40450 Selangor, Malaysia*
[2]*School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, 40450 Selangor, Malaysia*
[3]*School of Chemistry & Environment, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), Shah Alam, 40450 Selangor, Malaysia*
** Corresponding author E-mail: rohayaahmad@salam.uitm.edu.my*

Abstract

Muntingia calabura Linn. also known locally as "ceri kampung" is a plant of the family Elaeocarpaceae. The plant has been reported to possess several medicinal properties including reducing high blood pressure, lowering cholesterol level and controlling Type 2 diabetes. Type 2 diabetes is usually related to postprandial hyperglycemia, which is related to the rise of blood sugar level after a meal. This condition can be controlled by α-glucosidase inhibitors which inhibit the enzyme from catalyzing the liberation of glucose from carbohydrates in the digestive tract. Despite many biological studies reported for the plant, its antidiabetic potential has not been widely explored. Thus the aim of this study was to find potential α-glucosidase inhibitors from 16 extracts of *M. calabura* as a therapeutic approach in decreasing postprandial hyperglycemia. The hexane (Hx), ethyl acetate (Ea), 75% ethanol (Et) and aqueous (Aq) extracts of four parts (fruit, leaf, stem and root) of *M. calabura* (collected from Bangi, Selangor) were screened for their α-glucosidase inhibitory activities at 50.00, 25.00, 12.50, 6.25, 3.13, 1.56 and 0.78 ppm prepared via two-fold serial dilution against the positive control, acarbose. The aqueous leaf (AqL) and root extracts (AqR) exhibited very strong activities with IC_{50} values of 0.15 and 0.41 µg/ml while the other extracts showed strong to moderately strong activities with IC_{50} values ranging from 1.83-11.66 µg/ml against acarbose (4.3 µg/ml).

Keywords: *Muntingia calabura, antidiabetic, postprandial hyperglycemia, α-glucosidase*

1. Introduction

Muntingia calabura Linn. (family Elaeocarpaceae), one of the species in the genus Muntingia, is a flowering plant native to Southern Mexico, the Caribbean, Central America, and Western South America. The tree is easily growing and widespread. In Malaysia, it is well-known as "ceri kampung" or "Kerukup Siam" [1]. *Muntingia calabura* Linn. is rarely used among the Malay folklore medicine. However, it is traditionally used by the people in Peru to treat various diseases in the country [2]. Many medicinal advantages can be attained from this plant such as treating gout, curing diabetes, relieving flu symptoms and headaches, lowering high blood pressure, lowering cholesterol levels in the blood and also as antibacterial or antiseptic medicine [3]. Many studies have been done on this plant and it has been reported to be rich in flavonoid compounds which are believed to be effective against diabetes and other diseases.

Diabetes mellitus is a fast growing disease. In 1980, it was recorded that 108 million people suffer from this disease. Between 2009 and 2034, the number of people with diagnosed and undiagnosed diabetes is expected to increase from 23.7 million to 44.1 million [4]. Diabetes consists of two major types which are known as Type 1(T1D) and Type 2(T2D). In T1D also known as insulin-dependent diabetes, the pancreas undergoes an autoimmune attack by the body itself. In T2D or non-insulin-dependent diabetes, the insulin is produced continuously by the pancreas but the body has trouble using this glucose-controlling hormone, resulting in insufficient amount of insulin to meet the body's needs [5]. Here, the postprandial phase is characterized by a fast and large increase in blood glucose levels (hyperglycemic spikes) possibly relevant to the onset of cardiovascular complications. By delaying the degradation of carbohydrate via inhibition of the key hydrolyzing enzymes α-glucosidase or α-amylase, the absorption of glucose will decrease resulting in the reduction of postprandial hyperglycemia [6,7].

To date, selected parts of *M. calabura* has been investigated for their enzyme inhibitory properties (mostly focusing on α-glucosidase inhibitory studies). Some promising results have been reported in separate *in-vitro* or *in-vivo* studies for the leaves and the roots of the plant. However, no systematic or comparative study of the *in-vitro* activity of different parts of the plant has been carried out. Hence, with the aim of identifying the active extracts of the plant, we now report the *in-vitro* α-glucosidase inhibitory activities of 16 extracts comprising four different parts of *M. calabura* including its fruits, leaves, stems and roots.

2. Materials and method

2.1. Chemicals and raw materials

All chemicals used were of analytical grade and purchased from Sigma Chemical Co. (St Louis, Missouri) and were distilled prior to use. Fresh young fruits, leaves, stems and roots of *M. calabura* were collected in Bangi, Selangor, Malaysia in October 2016 and

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a voucher specimen was deposited in Putrajaya Herbarium. Each part of the plant was washed thoroughly with water to remove adhering dirt and dried under the shade. The completely dried samples were cut into small pieces. The fruits were freeze-dried while the other samples were ground to fine powder.

2.2. Extraction of fruits, leaves, roots and stems of *Muntingia calabura*

About 1kg of powdered sample was extracted consecutively with hexane (Hx), ethyl acetate (Ea), 75% ethanol (Et) for 3 days each successively. The aqueous (Aq) extract of each part of the plant was prepared by adding distilled water to the marc and sonicating it in a water bath at 60°C for 60 minutes. The supernatant was filtered via vacuum filtration and concentrated *in vacuo* using a rotary evaporator. To maintain its freshness and prevent from contamination, the crude extracts were tightly stored at 4°C before being subjected to the bioassay.

2.3. α-Glucosidase Inhibitory Assay

2.3.1. Preparation of reagent

Phosphate buffered saline was prepared by dissolving 1.4g of sodium phosphate monobasic, 8g of sodium chloride, 0.2g of potassium chloride and 0.2g potassium dihydrogen phosphate in 1L deionized water. The solution was adjusted to pH 6.5 at temperature 20˚C with 1M of sodium hydroxide, NaOH and hydrochloric acid, HCl. 20mM of *para*-nitrophenyl-α-D-glucopyranoside (p-NPG) substrate was prepared by dissolving 6mg p-NPG in 10ml of buffer solution. The α-glucosidase enzyme solution was prepared by dissolving α-glucosidase enzyme, type 1 (from Baker's yeast) 1mg in 1000µL cold phosphate buffered saline, pH 6.5. 50µL of solution was mixed into 12ml of cold buffered saline to give a concentration of 0.125unit/ml. For the screening of plant extracts, 100 µg/ml concentration was prepared in 5% dimethyl sulfoxide (DMSO) followed by two fold serial dilution to give concentration of 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 µg/ml. Acarbose as the positive control was prepared at a concentration of 100 µg/ml in 5% DMSO and two fold serial dilution yielded concentration of 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 µg/ml.

2.3.2. Method

10µL of the plant extracts, 20µL of α-glucosidase enzyme, 40µL of phosphate buffered saline at pH 6.5 and 20µL of deionized water were mixed with each well in a 96-cell microtiter plate. The mixture was pre-incubated at a temperature of 37˚C for 10 minutes. Then, 10µL of 20mM p-NPG solution was added into the mixture and absorbance at 0 minutes was measured at the wavelength of 405nm. The reaction mixture was incubated at temperature 37˚C for 30 minutes and the absorbance was measured. For negative control, the plant extract was replaced with 5% of DMSO and acarbose was used as positive control. Experiments were performed in triplicates. α-Glucosidase inhibitory activity was conducted based on the method described by Ahmad *et al.* [8]. The percent inhibition of α-glucosidase inhibitory activity was calculated using the following equation:

$$\%\text{Inhibition}=(A_{30min}-A_{0min})\text{control}-(A_{30min}-A_{0min})\text{exp})/(A_{30min}-A_{0min})\text{control}\times 100 \quad (1)$$

where A is the absorbance of mixture measured at 405 nm.

3. Results and discussion

3.1. Antidiabetic potential of *M. calabura* Linn. via α-glucosidase inhibitory activity

M. calabura has been reported to possess *in-vitro* α-glucosidase inhibitory activity of its roots and *in-vivo* antihyperglycemic activity of its leaves [9,10]. Our results support and augment some of these findings by showing that the root extracts of the plant exhibited highest percent inhibition towards α-glucosidase and moderate-strong activities for other parts. Figure 1 shows the α-glucosidase inhibitory activities of 16 extracts *M. calabura* against acarbose. All samples showed high percentage of α-glucosidase inhibition at a concentration of 50 ppm. The extracts with higher concentration exhibited higher α-glucosidase inhibition and as the concentration decreased, the α-glucosidase inhibition decreased. These imply that at higher concentrations, the active constituents were present in higher quantities.

3.2. IC_{50} value of acarbose

Acarbose, a common α-glucosidase inhibitors was used as the positive control in this study. At a concentration of 50 ppm, acarbose showed inhibition of 85.1% and it possessed an IC_{50} of 4.29 µg/mL, comparable to the value reported by Yadav [11]. However, in general, IC_{50} values for acarbose have been found to be dependent on the materials and method employed and it differs from one study to another. Hence, in this study, the α-glucosidase inhibitory activities of the samples were interpreted based on their IC_{50} values, relative to that of acarbose. Based on these values, the activity of acarbose is considered moderately strong in this study.

3.3. IC_{50} value of 16 extracts *of Muntingia calabura*

Table 1 shows the α-glucosidase inhibitory activity of 16 extracts of *M. calabura* against acarbose. The aqueous leaf and aqueous root extract have IC_{50} values <1.0 µg/mL compared to the other extracts and considered as very strong in terms of inhibitory activity.

Table 1: α-Glucosidase inhibitory activity of 16 extracts of Muntingia calabura

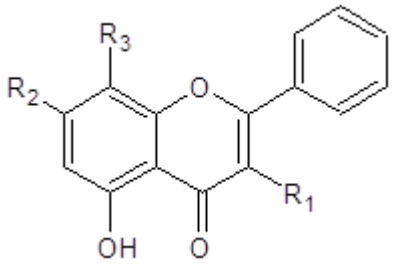
Extract	IC_{50} (µg/mL)	Activity
HxF	8.46±0.70	Strong
HxL	11.16±0.52	Moderately Strong
HxR	4.56±0.78	Strong
HxS	2.86±0.60	Strong
EaF	9.43±2.23	Strong
EaL	11.66±3.22	Moderately Strong
EaR	10.44±0.44	Moderately Strong
EaS	3.34±0.61	Strong
EtF	5.72±0.29	Strong
EtL	4.48±1.14	Strong
EtR	2.44±0.64	Strong
EtS	2.23±0.73	Strong
AqF	8.98±1.57	Strong
AqL	0.15±0.04	Very strong
AqR	0.41±0.26	Very strong
AqS	1.83±0.50	Strong
Acarbose	4.29±1.13	Strong

Very strong: < 1.0; Strong: 1.0-10.0; Moderately strong: 10.1- 50 µg/mL

3.4. Correlation between activity and constituents

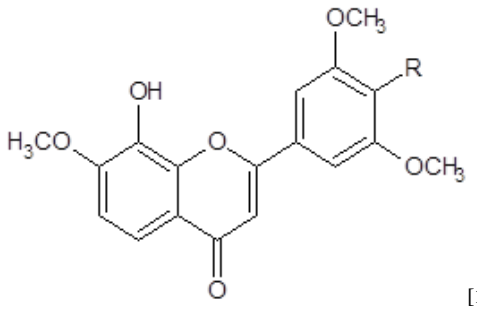
The effective treatment of diabetes is increasingly dependent on the active constituents of the medicinal plants that have capability of controlling hyperglycemia as well as its secondary complications [8]. Interestingly, Ramadas [10] reported that the *in-vitro* α-glucosidase inhibitory activity of the root extract of *M. calabura* (*IC*$_{50}$ value of 394.13 ±2.3 µg/mL) could be due to the presence of proteins. Phytochemical screening of *M. calabura* extracts indi-

cated the presence of various phytochemicals including flavonoids, tannins and saponins. The presence of these phytochemicals working in synergy may explain the observed antidiabetic potential. Flavonoids are commonly believed as antidiabetic agents from some plants. Figure 2 shows the structure of flavones and chalcone previously detected or isolated from the plant. These compounds may play important roles in the the inhibition against the α-glucosidase enzyme. However, to date, no single compound has been associated with the α-glucosidase inhibitory activity of the plant.



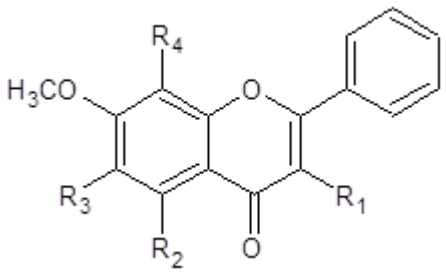
[12]

1 R_1= R_3=OMe, R_2=OH
2 R_1=R_2=OMe, R_3=H
3 R_1=R_2=OH, R_3=OMe



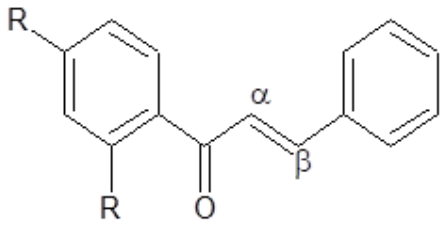
[13]

1 R=OMe
2 R=OH



[13]

1 R_1=R_4= H, R_2=OH, R_3=OMe
2 R_1=R_3=R_4=H, R_2=OMe
3 R_1=OH, R_2=OH, R_4=H, R_3=OMe



[12]

1 R=OH

4. Conclusions

The aqueous leaf and root extracts of *M. calabura* Linn. both exhibited very strong α-glucosidase inhibitory activities with IC_{50} of 0.15 and 0.41 μg/mL respectively which was stronger than the standard acarbose. All other 14 extracts exhibited from moderately strong to strong activities giving IC_{50} values in the range of 1.83-11.66 μg/mL. In conclusion, *M. calabura* extracts was found to possess potential natural α-glucosidase inhibitors which are able to suppress the conversion of polysaccharides into monosaccharides and hence capable of decreasing postprandial hyperglycemia. These activities may be attributed to the synergistic effects of various constituents including phytochemicals and proteins or individual constituents. Work is currently under way in identifying these active constituents.

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References

[1] M. I. M. Yusof, M. Z. Salleh, T. L. Kek, N. Ahmat, N. F. N. Azmin, and Z. A. Zakaria, "Activity-guided isolation of bioactive constituents with antinociceptive activity from *Muntingia calabura* L . leaves using the formalin test * Address all correspondence to : Associate Professor Dr . Zainul Amiruddin Zakaria Department of Biomedical Scien," *Hindawi*, vol. 2013, pp. 1–27, 2013.

[2] Z. A. Zakaria *et al.*, "Antinociceptive, anti-inflammatory and antipyretic effects of *Muntingia calabura* aqueous extract in animal models," *J. Nat. Med.*, vol. 61, no. 4, pp. 443–448, 2007.

[3] N. D. Mahmood *et al.*, *Muntingia calabura:* A review of its traditional uses, chemical properties, and pharmacological observations, vol. 52, no. 12. 2014.

[4] E. S. Huang, A. Basu, M. O'Grady, and J. C. Capretta, "Projecting the future diabetes population size and related costs for the U.S.," *Diabetes Care*, vol. 32, no. 12, pp. 2225–2229, 2009.

[5] A. B. Olokoba, O. A. Obateru, and L. B. Olokoba, "Type 2 diabetes mellitus: A review of current trends," *Oman Med. J.*, vol. 27, no. 4, pp. 269–273, 2012.

[6] A. Ceriello, "Postprandial Glucose Regulation and Diabetic Complications," *Arch. Intern. Med.*, vol. 164, no. 19, p. 2090, Oct. 2004.

[7] A. Banerjee, B. Maji, S. Mukherjee, K. Chaudhuri, and T. Seal, "In Vitro Antidiabetic and Anti-oxidant Activities of Methanol Extract of *Tinospora Sinensis*," *J. Appl. Biol. Biotechnol.*, vol. 5, no. 03, pp. 61–67, 2017.

[8] R. Ahmad *et al.*, "Antioxidant and Antidiabetic Potential of Malaysian Uncaria," *Res. J. Med. Plant*, vol. 5, no. 5, pp. 587–595, May 2011.

[9] M. Aruna Sindhe, Y. D. Bodke, and A. Chandrashekar, "Antioxidant and in vivo anti-hyperglycemic activity of *muntingia calabura* leaves extracts," *Der Pharm. Lett.*, vol. 5, no. 3, pp. 427–435, 2013.

[10] D. Ramadas, S. Chandrappa, H. R. Kashyap, A. Biotechnology, and M. Sciences, "*in Vitro* Anti-Diabetic Activity of *Muntingia*," vol. 4, no. 10, pp. 1526–1534, 2015.

[11] V. K. Yadav and A. Mishra, "In vitro & in silico study of hypoglycemic potential of *Pterocarpus marsupium* heartwood extract," *Nat. Prod. Res.*, vol. 6419, no. May, pp. 1–5, 2018.

[12] A. S. Sufian, K. Ramasamy, N. Ahmat, Z. A. Zakaria, and M. I. M. Yusof, "Isolation and identification of antibacterial and cytotoxic compounds from the leaves of *Muntingia calabura* L.," *J. Ethnopharmacol.*, vol. 146, no. 1, pp. 198–204, 2013.

[13] J. J. Chen, R. W. Lin, C. Y. Duh, H. Y. Huang, and I. S. Chen, "Flavones and cytotoxic constituents from the stem bark of *Muntingia calabura*," *J. Chinese Chem. Soc.*, vol. 51, no. 3, pp. 665–670, 2004.