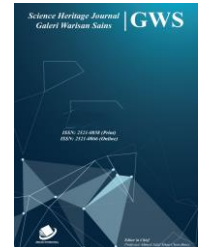


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RESEARCH ARTICLE

ANTI-DIABETIC POTENTIAL OF PEPTIDE FROM *P. NIRURI* REVEALS THROUGH CARBOHYDRATE HYDROLYZING ENZYME INHIBITION ASSAY

Noor Hasniza Md Zin*, Ainul Mardhiah Mohd Nail

Department of Biotechnology, Kulliyah of Science, International Islamic University Malaysia, Kuantan Campus, Jalan Istana, Bandar Indera Mahkota, 25200, Kuantan, Malaysia

*Corresponding author Email: hasnizamz@iium.edu.my

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ARTICLE DETAILS

ABSTRACT

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Herb *Phyllanthus niruri* (*P. niruri*) is known to have various pharmacological functions including anti-diabetic properties. In this research, protein extracts from different plant parts (leave, fruit and stem) of *P. niruri* were investigated for their anti-diabetic potential through α -amylase and α -glucosidase enzyme inhibition assays. For the enzyme inhibition assay, fruit was found to have the highest inhibition percentage (90.0%) against α -glucosidase followed by leave (62.6%) and stem (38.4%). The similar patterns were also recorded for the α -amylase enzyme inhibition assay, in which, fruit showed the highest inhibition percentage (64.1%) followed by leave (33.3%) and stem (18.2%). The findings of this research suggest that fruit of *P. niruri* is a potential plant part with regards to anti-diabetic properties since it exhibits the highest inhibition activity against both α -amylase and α -glucosidase enzyme compared to leave and stem.

KEYWORDS

Diabetes mellitus, *Phyllanthus niruri*, α -amylase, α -glucosidase.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder involving malfunctioning of the endocrine system and it is becoming the third world killer. DM can be classified into two types which are type 1 (insulin deficiency) and type 2 (insulin resistance) [1-3]. The World Health Organization reported that 347 million worldwide are suffering from DM and by the year 2030, WHO foresees that death cause by DM will be doubled up from the statistical records in the year 2005. Currently, many anti-diabetic drugs are available in the market such as sulfonylureas, biguanides and glinides [2]. Unfortunately, upon consuming, these drugs are showing toxicity effects and lead to other health complications to the patients. As an alternative and more natural approach, various medicinal plants have been used as the source of remedy including *P. niruri*.

P. niruri, locally known as "dukung anak" is a herbaceous plant belongs to Euphorbiaceae family. It originates from India and widely distributed in tropical climate. This plant grows well in moist, shady and sunny area. It is well known for having a great potential of nutraceutical and medicinal values. In Malaysia, *P. niruri* is traditionally used as hepatoprotective, anti-hypertensive and diuretic medicinal plant to treat jaundice, genital urinary infections and diarrhea [4-6]. A lot of researches have been done on *P. niruri* and proven that extract from different plant parts of this plant including leaves, fruits and roots contains various type of bioactive compounds such as flavonoids, alkaloids, terpenoids, lignin, coumrim and tannin which each of them serve for a particular pharmacological properties like antimicrobial, antibacterial, antioxidant and also anti-diabetic properties [7]. Hence, making *P. niruri* is a potential material for pharmaceutical industries. The trend in the screening of medicinal plants for antidiabetic activity has increased, as it is important to discover novel effective drugs for the disease [8].

Various mechanisms in managing DM have been investigated, including

retardation of activity of carbohydrate hydrolyzing enzymes [9,10]. Both α -amylase and α -glucosidase are enzymes that responsible for carbohydrate digestion in human body where α -amylase hydrolyzed the complex dietary carbohydrate, meanwhile α -glucosidase helps in glucose absorption from the small intestine to the blood stream [1,3]. The interference of enzymatic activity of both α -amylase and α -glucosidase would delay carbohydrates digestion which will contributes to lowered postprandial blood glucose level due to reduced rate of glucose absorption. In this study the inhibitory action of protein extracts from different plant parts of *P. niruri* were evaluated against α -amylase and α -glucosidase activity.

2. MATERIALS AND METHODS

2.1 Plant materials and protein extraction

The whole plant of *Phyllanthus niruri* was freshly collected from Taman Pertanian Jubli Perak Sultan Ahmad Shah, Kuantan. The plant was rinsed and separated into different plant parts (leaves, fruits and stems). Each plant part was analyzed in triplicate. Total soluble protein from different plant parts of *P. niruri* was extracted according to trichloroacetic acid (TCA)/acetone method [11]. Approximately, one gram of each plant part of *P. niruri* was ground in liquid nitrogen to a fine powder using mortar and pestle and then incubated in acetone containing 10% TCA (w/v) and 1% dithiothreitol (DTT) (w/v) for overnight at -20°C. The mixture was then centrifuged at 25,000 x g at 4°C for 20 minutes. The pellet obtained was then washed three times by suspension in acetone containing 1% (w/v) DTT and stored for 1 hour at -20°C. After that, the mixture was centrifuged at 25,000 x g at 4°C for 20 minutes and the pellet obtained was vacuum dried. Then, 2 ml of rehydration buffer comprising 8 M urea, 20 mM DTT, 4% (w/v) CHAPS and 2% (v/v) ampholyte (pH 3-10) was added to the vacuum dried pellet. The mixture was vortexed briefly and then stored at -20°C for 1 hour before being centrifuged at 25,000 x g at 4°C for 20 minutes. The supernatant was collected and the pellet was re-

suspended with the rehydration buffer and further centrifuged. The pellet was discarded and the collected supernatants were combined and then stored at -80°C for further proteomics analysis.

2.2 α-amylase inhibition assay

The inhibition activity of α-amylase using protein extract was determined following method proposed and slightly modified [12,13]. Protein extract (25 µg/ ml) was pre-mixed with 1ml of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing 0.5 mg/ml porcine α-amylase and incubated at 37°C for 5 minutes. After that, 1 ml of starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to the mixture and further incubated at 37°C for 10 minutes. Then, the catalytic reaction was terminated by the addition of 2 ml of dinitrosalicylic acid (DNS) color reagent while boiling the mixture in the 100°C water bath for 15 minutes. The mixture was left at room temperature to cool down before diluted with distilled water (5 ml). The absorbance of the reaction mixture was measured at 540 nm by using Lambda 35 UV-Vis spectrophotometer. Acarbose was used as the positive control. Meanwhile, 95% ethanol was used as a control replacing plant extract. For blank, enzyme solution was replaced with distilled water. The α-amylase inhibitory activity was expressed as inhibition percentage and calculated as follows:

$$\text{Inhibition percentage (\%)} = \frac{[A_{540} \text{ Control} - A_{540} \text{ Extract}]}{A_{540} \text{ Control}} \times 100(\%) \tag{1}$$

A₅₄₀ Control refers to absorbance read at 540 nm with α-amylase and starch. A₅₄₀ Extract refers to absorbance read at 540 nm with protein extract, α-amylase and starch.

2.3 α-glucosidase inhibition assay

The inhibitory activity of α-glucosidase using protein extract was determined following method proposed and slightly modified [12,13]. A protein extract (250 µl) was pre-incubated with 250 µl of 1.0 M potassium phosphate buffer, pH 6.9 containing 0.1 U/ml *Saccharomyces cerevisiae* α-glucosidase at 37°C for 2 minutes. After that, 250 µl of p-nitrophenyl-D-glucopyranoside in 1.0 M phosphate buffer, pH 6.9 was added to the pre-incubated mixture and further incubated at 37°C for 30 minutes. Then, 2 ml of 0.1 M sodium carbonate (Na₂CO₃) solution was added to the mixture to stop the catalytic reaction. The absorbance of the mixture and also a control consisting 250 µl of distilled water in place of protein extract was measured at 405 nm using Lambda 35 UV-Vis spectrophotometer. Acarbose was used as the positive control. Meanwhile, 95% ethanol was used as a control replacing plant extract. For blank, enzyme solution was replaced with distilled water. The α-glucosidase inhibitory activity was then expressed as inhibition percentage and calculated as follows:

$$\text{Inhibition percentage (\%)} = \frac{[A_{405} \text{ Control} - A_{405} \text{ Extract}]}{A_{405} \text{ Control}} \times 100 \tag{2}$$

A₄₀₅ Control refers to absorbance read at 405 nm with α-glucosidase and p-nitrophenyl-D-glucopyranoside.

A₄₀₅ Extract refers to absorbance read at 405 nm with protein extract, α-glucosidase and p-nitrophenyl-D-glucopyranoside.

2.4 Statistical analysis

All assays were performed in triplicates and the values were averaged. The data expressed in mean ± SEM. By using IBM SPSS Statistics 20 software, the homogeneity test of data was performed before proceeding to parametric test which is One Way Anova and post-hoc Tukey in order to

detect the significant differences between the data of different plant parts of *P. niruri*. The data are considered statistically significant when the P<0.05.

3. RESULTS AND DISCUSSIONS

Protein extracts from different plant part of *P. niruri* were examined against α-glucosidase and α-amylase to see the inhibitory activity towards both enzymes. Reaction between enzyme and substrate will results in formation of products in the system. The presence of inhibitor in the reaction will eventually reduce the amount of product released. In this study, enzyme α-glucosidase was reacted with p-nitrophenyl glucopyranoside (pNPG) while α-amylase reacted with starch substrate in the presence of potential inhibitor from different plant parts of *P. niruri*. The amount of reducing sugar (maltose) produced at the end of reaction was then quantified spectrophotometrically and tabulated in Table 1 below. Acarbose which is a known carbohydrate hydrolyzing enzyme inhibitor was used as the positive control.

Table 1: Maltose concentration (mean ± SEM) generated from α-glucosidase and α-amylase enzyme inhibition assay using acarbose and different plant parts of *P. niruri* as an inhibitor

Plant parts	Maltose Concentration (µg/µl)	
	α-Glucosidase	α-Amylase
Acarbose (control)	0.039±0.01	0.039±0.01
Leaves	0.655±0.11	0.959±0.06
Fruits	0.392±0.04	0.651±0.05
Stems	0.908±0.10	1.110±0.04

Table 1 shows the liberated reducing sugar from the reaction of carbohydrate hydrolyzing enzyme inhibition assays. In α-glucosidase enzyme inhibition assay, despite the presence of stem as an inhibitor in the reaction, the amount of maltose formation were found significantly compared to the positive control, acarbose. Following that were leave and fruit. Similar patterns of reducing sugar production also can be seen in α-amylase enzyme inhibition assay. The incorporation of stem and leave that show the inhibitory activities which showed significantly high amount of reducing sugar production. On the other hand, fruit produced a significantly lowest generation of reducing sugar compared to the other two plant parts.

Generally, high reducing sugar production indicates the low inhibitory activity in the reaction and vice versa. The observation on the differences in the amount of reducing sugar generated when different plant parts of *P. niruri* were employed during the reaction shows that this plant has a potential to disturb the action of this α-glucosidase and α-amylase enzyme activity during carbohydrates digestion by which fruit was found to possess the highest inhibition potential, followed by leave and stem. The percentage of inhibition of each plant part against α-glucosidase and α-amylase activity was displayed in Figure 1 below.

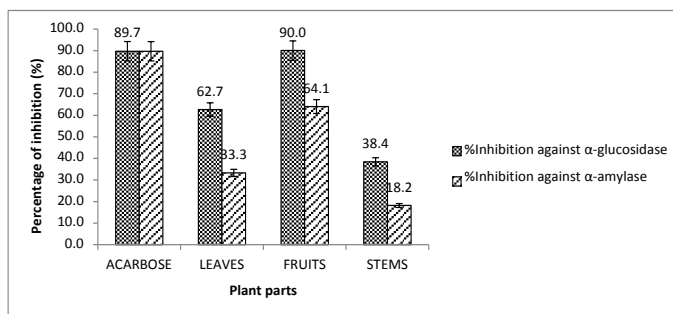


Figure 1: Inhibition percentage of different plant parts of *P. niruri* against

Figure 1 demonstrated the percentage of α-glucosidase and α-amylase inhibitory activity of these enzymes with the presence of potential inhibitor of different plant parts of *P. niruri* and acarbose. It can be seen that fruit showed the highest inhibitory activity (90.0%) against α-

glucosidase enzyme compared to leave (62.6%) and stem (38.4%). Fruit also exhibited almost similar inhibitory percentage activity as acarbose (89.7%) which is a known carbohydrate hydrolyzing enzymes inhibitor. This finding could suggest that the effectiveness of fruit as an inhibitor for α -glucosidase might be as good as acarbose compared to leave and stem. Meanwhile, similar pattern of inhibition percentage was also obtained against α -amylase. Fruit exerted the highest inhibition percentage (64.1%), followed by leave (33.3%) and stem (18.2%).

The findings of this study displayed that the protein extract from all three plant parts of *P. niruri* have shown inhibitory activity towards both α -glucosidase and α -amylase enzymes. Other than that, it also can be seen that the protein extracts shared similar patterns of inhibitory activity for both α -glucosidase and α -amylase enzymes where fruit showed the highest inhibition, followed by leave and stem. This in vitro study has demonstrated that *P. niruri* possess higher inhibition against α -glucosidase compared to α -amylase. This result is in parallel with the characteristic of interest in finding an ideal inhibitor for carbohydrate hydrolyzing enzymes. An excessive inhibition of enzyme α -amylase in the pancreas will cause abnormal bacterial fermentation of undigested carbohydrates in the colon [1,14,15]. Thus, natural inhibitor with strong α -glucosidase and mild α -amylase inhibition is current interest.

4. CONCLUSION

In conclusion, the inhibition activity of each protein extracts from different plant parts of *P. niruri* toward both carbohydrates hydrolyzing enzymes; α -glucosidase and α -amylase activity were successfully studied. The similar pattern of inhibition activity by the protein extracts can be seen for both enzymes, however, differ in inhibition percentage was observed. *P. niruri* has shown a higher inhibition against α -glucosidase compared to α -amylase. Other than that, fruit was found to be a potent plant part of *P. niruri* with regard to its anti-diabetic properties since has shown the highest inhibition activity against both α -glucosidase and α -amylase enzymes.

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REFERENCES

- [1] Kwon, Y.I., Apostolidis, E., Kim, Y.C., Shetty, K. 2007. Health Benefits of Traditional Corn, Beans, and Pumpkin: In Vitro Studies for Hyperglycemia and Hypertension Management. *Journal of Medicinal Food*, 10 (2), 266–275.
- [2] Azwa, N., Wahab, A., Abdullah, N., Aminudin, N. 2014. Characterization of Potential Antidiabetic-Related Proteins from *Pleurotus pulmonarius* (Fr.) Quél. (Grey Oyster Mushroom) by MALDI-TOF / TOF Mass Spectrometry.
- [3] Oboh, G., Akinyemi, A.J., Ademiluyi, A.O., Bello, F.O. 2013. Inhibition of α - amylase and α -glucosidase activities by ethanolic extract of *Amaranthus cruentus* leaf as affected by blanching, 7 (17), 1026–1032.
- [4] Naik, A.D., Juvekar, A.R. 2003. Effects of alkaloidal extract of *Phyllanthus niruri* on HIV replication. *Indian Journal of Medical Sciences*, 57, 387–393.
- [5] Okoli, C.O., Ibiem, Akah, P.A., Okoye, T.C. 2010. Evaluation of antidiabetic potentials of *Phyllanthus niruri* in alloxan diabetic rats. *African Journal of Biotechnology*, 9 (2), 248–259.
- [6] Satya, A., Narendra, K. 2012. *Phyllanthus niruri*: A Review on its Ethno Botanical, Phytochemical and Pharmacological Profile. *Journal of Pharmacy*, 5 (9), 4681–4691.
- [7] Lee, N.Y.S., William, K.S.K., Mohammad, A.A., Tanes, P.M., Anne, R.F., Kamalan, J. 2016. The pharmacological potential of *Phyllanthus niruri*. *Journal of Pharmacy and Pharmacology*, 68, 953–969
- [8] Wickramaratne, M.N., Punchihewa, J.C., Wickramaratne, D.B. 2016. In-vitro alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*. *BMC complementary and alternative medicine*, 16 (1), 466.
- [9] Kumar, A., Bharti, S.K., Kumar, A. 2017. Therapeutic molecules against type 2 diabetes: What we have and what are we expecting? *Pharmacological Reports*, 69 (5), 959–970. <http://doi.org/10.1016/j.pharep.2017.04.003>
- [10] Upadhyay, J., Polyzos, S.A., Perakakis, N., Thakkar, B., Paschou, S.A., Katsiki, N., Mantzoros, C.S. 2018. Pharmacotherapy of type 2 diabetes: An update. *Metabolism: Clinical and Experimental*, 78, 13–42.
- [11] Sheoran, I.S., Ross, A.R.S., Olson, D.J.H., Sawhney, V.K. 2009. Compatibility of plant protein extraction methods with mass spectrometry for proteome analysis. *Plant Science*, 176, 99–104. <http://doi.org/10.1016/j.plantsci.2008.09.015>
- [12] Apostolidis, E., Kwon, Y., Shetty, K. 2005. Potential of cranberry-based herbal synergies for diabetes and hypertension management. *Asia Pacific Journal of Clinical*, 15, 433–441.
- [13] Wahab, N., Abdullah, N., Aminudin, N. 2014. Characterisation of Potential Antidiabetic-Related Proteins from *Pleurotus pulmonarius* (Fr.) Quél. (Grey Oyster Mushroom) by MALDI-TOF/TOF Mass Spectrometry. *BioMed Research International*.
- [14] Kazeem, M.I., Adamson, J.O., Ogunwande, I.A. 2013. Modes of inhibition of α -amylase and α -glucosidase by aqueous extract of *Morinda lucida* benth leaf. *BioMed Research International*.
- [15] Apostolidis, E., Kwon, Y. I., Shetty, K. 2006. Potential of select yogurts for diabetes and hypertension management. *Journal of Food Biochemistry*, 30, 699–717.

