The Potency of Red Betel (Piper crocatum Ruiz & Pav.) Methanolic Extract as α-Amylase and α-Glucosidase Inhibitor

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The Potency of Red Betel (*Piper crocatum* Ruiz & Pav.) Methanolic Extract as α-Amylase and α-Glucosidase Inhibitor

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ABSTRACT

Methanol is an effective menstruum for attracting compounds with various pharmacological activities from *Piper crocatum* Ruiz & Pav. Several studies reported that laboratory tests for reducing blood sugar content of red betel extract such as aqueous, ethanolic, and ethyl acetate extracts. This study aimed to investigate the actions of red betel leaves methanolic extract in reducing α-amylase and α-glucosidase in an *in-vitro* study. The test for decreasing the actions of the enzymes was done using the ultraviolet-visible spectrophotometric method. The test was run by observing carbohydrates left by knowing the effect of iodine-iodide resulting in a blue complex. The red betel leaves extract demonstrated an adequate α-amylase and α-glucosidase inhibitory activity as shown by the IC_{50} of 8.463 ± 0.318 mg/mL and 10.013 ± 0.070 mg/mL, respectively.

Keywords: *α-amylase, α-glucosidase, Methanolic extract, Piper crocatum.*

1. INTRODUCTION

It has been reported that some medicinal plants species have hypoglycaemic activity. Most plants contain bioactive components, such as polyphenols, alkaloids, terpenoids, flavonoids, coumarins, and other constituents [1]. Variations of phytochemical classes and bioactive compounds' chemical structure may differ in action mechanisms on reducing blood glucose [2]. The imbalance between blood sugar absorption and insulin production resulted in diabetes mellitus type 2.

Controlling blood sugar levels could be done by inhibiting the activity of hydrolytic enzymes that digest dietary starch, such as α-amylase and α-glucosidase enzymes. Methanol, ethanol, or a mixture of alcohol and water are common solvents used to extract bioactive compounds from the plant, also known as the menstruum [3,4]. The literature reported that methanol is an effective menstruum for attracting compounds with various pharmacological activities from red betel (*Piper crocatum* Ruiz & Pav.). Furthermore, Pandithurai *et al.*[7] found a methanolic extract of marine brown algae showed the reduction of α-amylase and α-glucosidase actions [5,6,7]. Several studies reported that the laboratory test of diabetic medicines from *P. crocatum* compounds such as aqueous, ethanolic, and ethyl acetate extracts [8,9,10,11].

The study aimed to evaluate the antidiabetic potency of red betel leaves methanolic extract activity through the α-amylase and α-glucosidase inhibition and identify the extracted compound. Methanol is expected to be able to extract antidiabetic compounds from red betel. The success of the extraction of these antidiabetic compounds was measured by the ability of *P. crocatum* methanol extract to reduce the actions of the α-amylase and α-glucosidase enzymes.

2. METHODOLOGY

The maceration method was used in the compound extraction of red betel leaves. The red betel leaves methanolic extract compounds profile was checked using Thin Layer Chromatography (TLC). Compound separation of the extracts has been detected by three types of mobile phase composition; n-hexane:ethyl acetate (3:1), chloroform:ethyl acetate (1:1), and toluene:ethyl

acetate (2:1). Spots detection of the separated compounds was carried out by spraying Lieberman Burchard's reagent, cerium sulfate, iodine, $FeCl_3$, and observing under UV-Visible spectrophotometer (254 nm and 366 nm). Assay of α-amylase enzyme inhibitory activity was done following Ononamadu *et al.* [12]. The potato powder (1% w/v), 1.0 ml extract/1 ml acarbose, 1.0 ml of the α-amylase enzyme (1% w/v), and 2.0 ml of acetate buffer (0.1M, 7.2 pH) were blended together. Inhibitory effect of the sample blank solution measurement was done by pouring 1.0 ml of 0.5% (w/v) potato starch solution into a test tube. After one hour incubation of the mixture, a 0.1 ml iodine-iodide was then poured.

The α-amylase enzyme inhibitory activity test was done following Pandhithurai *et al.* [7] with slightly modified. Extract and standard solutions of 100.0 µL from each series of test solutions were added with 400.0 µL of phosphate buffer and 250.0 µL of maltose substrate solution into a test tube and then pre-incubated at 37 °C for 5 minutes. After pre-incubation completion, added 250.0 µL of phosphate buffer solution pH 7.0 and then homogenized. Then the solution was incubated for 30 minutes at 37 °C. 0.3 mL of the solution was then taken, and 0.3 mL of DNS reagent was added to the test tube. The solution was homogenized and heated in boiling water for 5 minutes. The solution is mixed by adding 3.0 mL of distilled water. The absorbance was detected using a UV-vis spectrophotometer at 536 nm. The percent activity reduction was counted using Equation (1), where Ac means the absorbance provided by the control experiment, while As is the tested sample absorbance

$$\% \text{ inhibition} = (As\text{-}Ac/As) \times 100 \quad (1)$$

The results were statistically evaluated for ANOVA ($p < 0.05$) and continued by the Tukey's Post-Hoc Test (α=0.05) using an IBM SPSS version.

3. RESULTS AND DISCUSSION

Red betel leaves methanolic extract inhibited hydrolytic enzymes, both α-amylase and α-glucosidase. The red betel leaves extract significantly increased enzyme inhibition activity against the α-amylase enzyme ($P<0.05$). The red betel percent inhibition of α-amylase was found to range from 19.44 to 55.56%, while the percent inhibition of α-glucosidase was found to range from 12.24 to 49.02% (Figure 1). The values of their IC_{50} for both α-amylase and α-glucosidase reduction due to the extract of *P. crocatum* leaves were 8.463 ± 0.318 mg/mL and 10.013 ± 0.070 mg/mL, respectively. As the positive control used in this study, acarbose showed IC_{50} value was 0.837 ± 0.076 mg/mL for α-amylase inhibitory and 0.690 ± 0.124 mg/mL for α-glucosidase inhibitory activities. It means that the acarbose elicited greater percentage inhibition value than *P. crocatum* leaves methanolic extract for inhibiting the α-amylase and α-glucosidase. The IC_{50} value of acarbose was. In acarbose, the concentration to reduce α-amylase was greater than those of α-glucosidase, but the opposite occurs in P. *crocatum* leaves methanolic extract.

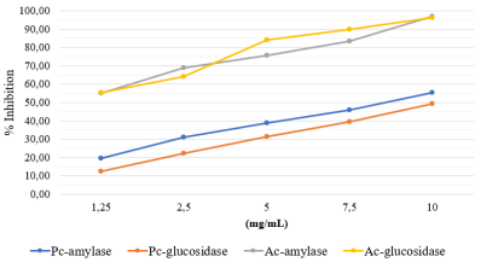

Figure 1. Percent enzymes inhibition activity of *P. crocatum* methanolic extract and acarbose: Pc-amylase: *P. crocatum* methanolic extract against α-amylase enzyme; Pc-glucosidase: *P. crocatum* methanolic extract against α-glucosidase enzyme; Ac-amylase: Acarbose against the α-amylase enzyme, and Ac-glucosidase: Acarbose against the α-glucosidase enzyme.

Several researchers tested the antidiabetic activity of plants against α-amylase or α-glucosidase separately or both [9, 11, 13, 7]. It has been observed that the ethanolic extract of 14 medicinal plants reduced the activities of α-amylase and α-glucosidase, and α-amylase was not affected by plant extract concentration [13]. The same compound did not reduce α-amylase action as occurred in α-glucosidase. The compound mixture from plant extracts could act synergist, additive, or antagonistic. If the mixture compounds interact synergistically, it seems that they are more effective than those of pure compounds [14].

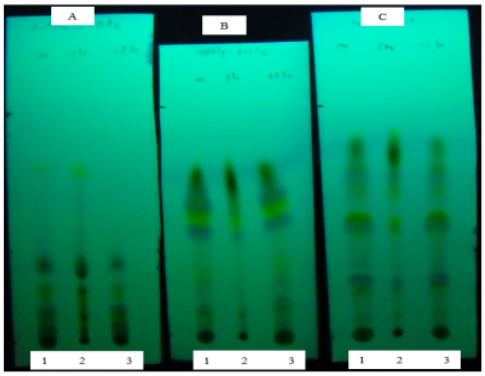

Figure 2. Thin Layer Chromatogram of *P. crocatum* methanolic extract observed under 254 nm (1), extract soluble in n-hexane (2), extract insoluble in n-hexane (3) in mobile phase compostion of n-hexane:ethyl acetate (3:1) [A], chloroform:ethyl acetate (1:1) [B], and toluene:ethyl acetate (2:1)[C].

The thin layer chromatography mobile phase composition in separating process of compounds in red betel leaves methanolic extract were n-hexane:ethyl acetate (3:1); chloroform:ethyl acetate (1:1) and toluene:ethyl acetate (2:1). Each mobile phase type used in this study separated the compounds in the extract (Figure 2). Further fractionation of the methanol extract was carried out using n-hexane. Some of the red betels leaves methanolic extract compounds were extracted in the soluble and insoluble fractions of n-hexane. However, in general, the n-hexane solvent was quite effective in separating these compounds.

In addition to methanol, compounds extracted with other polar solvents such as water showed antidiabetic activity from the extract. Some phenolic compounds, namely cyanidin 3-O-glucoside, caffeic acid, tannin, p-coumaric acid, and gallic acid are identified for antidiabetic activity [10]. Hartini *et al.* [5] fractionated methanol extract using vacuum liquid chromatography with various compositions of n-hexane and ethyl acetate, detected the presence of essential oils, tannins, alkaloids, terpenoids, and flavonoids. Tannin has been reported as an α-amylase and α-glucosidase enzyme inhibitor [13, 15]. Tropical almonds, petroleum ether, and methanolic extracts are rich in tannins with antidiabetic activity [16]. Chemically tannins are complex substances, and they usually occur in mixtures of polyphenol that are difficult to separate because they do not crystallize.

According to Barret *et al.* [15], the effectiveness of tannins in inhibiting the enzyme's activity was affected by molecule size and structural complexity. In addition to tannin, other groups of compounds reduced enzyme activity. The ethyl acetate fraction of red betel leaves extract contains the terpenoid (Columbine), which was stronger in inhibiting the activity of the α-glucosidase enzyme than other compounds [9]. Many factors seem to affect extract activity in reducing α-glucosidase, i.e., harvesting time, storage conditions, cultivation, climate, processing, and genetic background [10]. Red betel leaves methanolic extract can be fractionated with non-polar solvents such as n-hexane to obtain the compound responsible for the reduction of α-amylase and α-glucosidase enzymes activities. In view of tannins' reported solubility and activity, further research needed on the activity of compounds in the insoluble n-hexane fraction and the soluble n-hexane fraction in inhibiting the activity of -amylase and -glucosidase enzymes.

The antidiabetic ingredients of red betel leaves can be extracted using methanol. Red betel leaves methanolic extract demonstrated inhibitory activities for the α-amylase or α-glucosidase enzyme. Fractionation using n-hexane as solvent effectively separated the compounds in red betel leaves methanolic extract. It needs further investigation to confirm the activity of the soluble and insoluble fractions of n-hexane to ascertain which compound or group of compounds are responsible for the reduction of α-amylase or α-glucosidase enzymes activity from red betel leaves methanolic extract.

AUTHORS' CONTRIBUTIONS

Yustina Sri Hartini was responsible for the whole experiment, including expressing the idea and designing the experiments, preparing material, reagents, tools, performing the experiments, and writing the paper. Dewi Setyaningsih contributed to reagents provision, performing the experiment, analyzing, and interpreting the data.

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REFERENCES

[1] D.K. Patel, S.K. Prasad, R. Kumar, S. Hemalatha, An overview on antidiabetic medicinal plants having insulin mimetic property, Asian Pac. J. Trop. Biomed vol. 2, 2012, pp. 320-330. DOI: https://doi.org/10.1016/S2221-1691(12)60032-X

[2] O. Patience, Osadebe, U. Estella, Odoh, F. Philip, Uzor, The search for new hypoglycemic agents from plant, African Journal of Pharmacy and Pharmacology vol 8, 2014, pp. 292-303. DOI: https://doi.org/10.5897/AJPP2014.3933

[3] S. Sasidharan, Y. Chen, D. Saravanan, K.M. Sundram, L.Y. and Latha, Extraction, Isolation and Chareterixzation of Bioactive Compound from Plants' Extracts, Afr. J Tradit Complement Altern Med vol. 8, 2011, pp. 1-10. DOI: 10.4314/ajtcam.v8i1.60483

[4] A.R. Abubakar and M. Haque, Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes, Journal of Pharmacy & Bioallied Sciences vol. 12, 2020, pp. 1-10.DOI: https://doi.org/10.4103/jpbs.JPBS_175_19

[5] Y.S. Hartini, S. Wahyuono, S. Widyarini, A. Yuswanto, Phagocytic Macrophage Activity of Fractions form Methanolic Leaf Extract of Red Betel (*Piper crocatum* Ruiz & Pav.) In Vitro, Jurnal Ilmu Kefarmasian Indonesia vol. 11, 2013, pp. 108-115. DOI:https://doi.org/10.4314/tjpr.v13i10.6

[6] B.D. Wicaksono, Y.A. Handoko, E.T. Arung, I.W. Kusuma, D. Yulia, A.N. Pancaputra, Antiproliferative effect of metanol extract of *Piper crocatum* Ruiz & Pav leaves on human breast (T47D) cells *in vitro*, Tropical Journal of Pharmaceutical Research vol. 8, 2009, pp. 345-352. DOI: https://doi.org/10.4314/tjpr.v8i4.45229

[7] M. Pandithurai, S. Murugesan, S. Bhuvaneswari, S. and Thennarasan, In vitro α-amylase and α-

glucosidase inhibition activity of methanolic extract of marine brown alga *Spatoglossum asperum*. International Journal of Advances in Pharmaceutics vol. 4, 2015, pp. 83-87. DOI: https://doi.org/10.7439/ijap.

[8] M. Safitri and F. Fahma, 2008 Potency of *Piper crocatum* Decoction as an Antihiperglycemia in Rat Strain Sprague dawley, Hayati Journal of Biosciences vol. 15, 2008, pp. 45-48. DOI: https:doi.org/10.4308/hjb.15.1.45

[9] M. Weni, M. Safithri, and D.S.H. Seno, 2020 Molecular Docking of Active Compounds *Piper crocatum* on The Alpha-Glucosidase Enzyme as Antidiabetic, Indonesian Journal of Pharmaceutical Science and Technology, vol. 7, No. 2, 2020, pp. 64-72. DOI:https://doi.org/10.24198/ijpst.v7i2.21120

[10] S.R.S. Kamaruzaman, K.F. Kasim, and M.N. Jaafar, The Effect of Harvesting Time on the Antioxidant and Antidiabetic Activity of *Piper crocatum* (Sirih Merah) Extract. IOP. Series: Material Science and Engineering vol. 864, IOP Publishing, 2020. DOI:10.1088/1757-899X/864/1/012211.

[11] Y.S. Hartini, D. Setyaningsih, M.J.V. Chang, and M.C.I.A. Nugrahanti, Sambiloto (*Andrograpis paniculata* Nees) leaf extract activity as an α-mylase enzyme inhibitor. Pharmacy Education vol. 21, 2021, pp. 305-308. DOI: https://doi.org/10.46542/pr.2021.212.305308

[12] C.J. Ononamadu, O.C. Ezeigwe, T.A. Owolarafe, G.O. Ihegboro, T.A. Lawal, K. Salawu, U.F. Umeoguaju, and I. Aminu, Starch-iodine assay method underestimates -amylase inhibitory potential of antioxidative compound and extracts. BioTechnologia vol. 101, 2020, pp. 45-54. DOI:http://doi.org/10.5114/bta.2020.93103

[13] B. Elya, R. Handayani, R. Sauriasari, U.S. Hasyyati, I.T. Permana, and Y.I. Permatasari, Antidiabetic Activity and Phytochemical Screening of Extracts from Indonesian Plants by Inhibition of Alpha Amylase, Alpha Glukcosidase and Dipertidyl Peptidase IV, Pakistan Journal of Biological Sciences vol. 18, 2015, pp. 279-284. DOI: http:doi.org/10.3923/pjbs.2015.279.284

[14] L.K. Caesar and N.B. Cech, Synergi and Antagonism in Natural Product Extracts: When 1+1 does not equal 2, Natural Product Reports vol. 36, 2019, pp. 845-936. DOI: http:doi.org/10.1039/c9np0011a

[15] A. Barret, T. Ndou, C.A. Hughey, C. Straut, A. Howell, Z. Dai, and G. Kaletunc, Inhibition of α-amylase and glucoamylase by tannins extracted from cocoa, pomegranates, cranberries and grapes. J. Agric. Food Chem. vol. 61, 2013, pp. 1477-1486. DOI:http:doi.org/10.1021/jf304876g

[16] A.N. Nagappa, P.A. Thakurdesai, R.N. Venkat, S. Jiwan, Antidiabetic activity of *Terminalia catappa* Linn fruits, J Ethnopharmacol vol. 88, 2003, pp. 45-50.DOI:http/doi.org/10.1016/s0378-8741(03)00208-3

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