Title: 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 dan α-Glucosidase Inhibitor

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ABSTRACT

Methanol is an effective menstruum for attracting compounds with various pharmacological activities from Red betel (*Piper crocatum* Ruiz & Pav.). Several studies reported that *in vitro* and *in vivo* antidiabetic activity of red betel extract, such as: aqueous, ethanolic, and ethyl acetate extracts. The aim of this study was to investigate the red betel leaf methanolic extract inhibitory activity against α-amylase and α-glucosidase enzymes. The inhibitory activity test of α-amylase and glucosidase enzymes was carried out using the ultraviolet-visible spectrophotometric method by measuring the absorbance of the remaining starch which forms a blue complex with iodine-iodide. The methanolic extract of red betel leaf showed both α-amylase and α-glucosidase inhibitory activity with IC_{50} values of 8.463 ± 0.318 mg/mL and 10.013 ± 0.070 mg/mL, respectively.

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

1. INTRODUCTION

It has been reported some medicinal plants species having hypoglycaemic activity, most plants contain bioactive components, such as polyphenols, alkaloids, terpenoids, flavonoids, coumarins and other constituents [1]. The variety of phytoconstituents classes and the wide differences, in the molecular structure of the isolated compounds suggest the possibility of different mechanisms of action on lowering blood glucose [2]. The type 2-diabetes mellitus is caused by an imbalance between blood sugar absorption and insulin secretion. Inhibiting the activity of hydrolytic enzymes that digest dietary starch, such as α-amylase and α-glucosidase enzyme are strategies for control blood sugar levels. Methanol, ethanol, or mixture of alcohol and water are common solvents used for extraction of bioactive compounds from plant, also known as the menstruum [3-4]. Methanol is an effective menstruum for attracting compounds with various pharmacological activities from red betel (*Piper crocatum* Ruiz & Pav.) andmarine brown algae extracts that inhibit α-amylase and α-glucosidase enzymes [5-7]. Several studies reported that both *in vitro* and *in vivo* antidiabetic activity of red betel extract, such as: aqueous, ethanolic, and ethyl acetate extracts [8-11]. The aim of this study was to investigate the red betel leaf methanolic extract antidiabetic activity against α-amylase and α-glucosidase enzymes and identify the extract compound. The level of inhibitory activity of α-amylase and α-glucosidase from red betel methanolic extract showed the effectiveness of selecting methanol as a solvent for antidiabetic compounds.

2. METHODOLOGY

Maceration method was used in the compound extraction of red betel leaf. Identification of red betel leaf methanolic extract compounds was carried out using Thin Layer Chromatography (TLC). Compound separation of the extracts have been detected by 3 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). The α-amylase enzyme inhibitory activity test was carried out according to Ononamadu *et al.* [12] with minor modifications. The potato starch (1% w/v), 1 ml of test material (extract, acarbose), 1 ml of α-amylase enzyme (1% w/v) and 2 ml of acetate buffer (0,1M, 7,2 pH) were mixed. The measurement of the inhibitory effect of the sample blank solution was carried out by taking 1 ml of 0.5% potato starch solution into a test tube. The mixture was incubated for one hour, then a 0.1 ml iodine-iodide indicator was added to the mixture. α-amylase enzyme inhibitory activity test was carried out according to Pandhithurai *et al.* [7]. Extract and standard solutions of 100 L from each series of test solutions were added with 400 L of phosphate buffer and 250 L of maltose substrate solution into a test tube and then pre-incubated at 37 °C for 5 minutes. After completion of pre-incubation, added with 250 L of phosphate buffer solution pH 7.0 and then homogenized. Then, the solution was incubated for 30 minutes at 37 °C. Then, 0.3 mL of the solution was 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 mL of distilled water. The absorbance measurement used UV-vis spectrophotometer using a wavelength of 536 nm. The percentage inhibition calculations did as follow:

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

Ac is the absorbance of the control; As is the absorbance of the sample.

To compare the treatment, this research used the analysis of variance (ANOVA) and $p < 0.05$ received the result of statistically significant, alongside the Tukey's Post-Hoc Test (α=0.05).

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3. RESULTS

Red betel leaf methanolic extract inhibit hydrolytic enzymes, both α-amylase and α-glucosidase. The inhibitory activity against amylase enzyme was found significantly increase ($P<0.05$) with concentration of red betel leaf methanolic extract. The red betel percent inhibition of α-amylasewas 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 methanolic extract of red betel leaf showed both α-amylase and α-glucosidase inhibitory activity with IC_{50} values of 8.463 ± 0.318 mg/mL and 10.013 ± 0.070 mg/mL, respectively. Acarbose as the positive control used in this study showed greater percentage inhibition than red betel leaf methanolic extract both on α-amylase and α-glucosidase inhibitory activity. The IC_{50}

value of acarbose was 0.837 ± 0.076 mg/mL for α-amylase inhibitory and 0.690 ± 0.124 mg/mL for α-glucosidase inhibitory activity.

In acarbose, the required concentration to inhibit the α-amylase enzyme activity was higher than to inhibit α-glucosidase enzyme activity, but the opposite happens to red betel leaf methanolic extract.

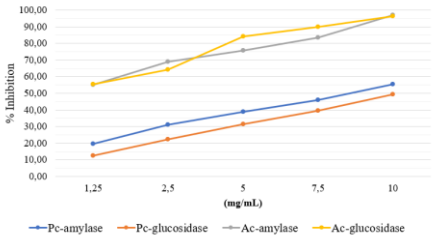

Figure 1 Percent enzymes inhibition activity of red betel methanolic extract and acarbose: Pc-amilase: Red betel methanolic extract against α-amylase enzyme; Pc-glucosidase: Red betel methanolic extract against α-glucosidase enzyme; Ac-amylase : Acarbose against α-amylase enzyme, and Ac-glucosidase: Acarbose against 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, ethanolic extract of 14 medicinal plants have α-amylase and α-glucosidase inhibitory activities, and α-amylase were not depended on concentration [13]. The inhibitory activity of the α-amylase enzyme was not produced by the same compounds as those that inhibited α-glucosidase. The overall activity of plant extracts can result from mixtures of compounds with synergistic, additive, or antagonistic activity. It seemed that they were more effective than purified compounds due to beneficial "synergistic" interactions [14].

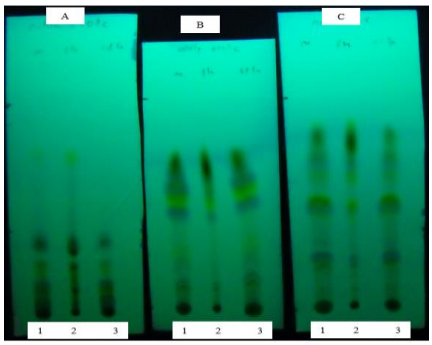

Figure 2 Thin Layer Chromatogram (TLC) of red betel methanolic extract [1.], extract soluble in n-hexane [2], extract insoluble in n-hexane [3] using mobile phase: n-hexane:ethyl acetate (3:1) [A], chloroform:ethyl acetate (1:1) [B], and toluene:ethyl acetate (2:1)[C].

The process of separating compounds in red betel leaf methanolic extract using three Thin Layer Chromatography mobile phase compositions, namely 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 was able to separate the compounds in the extract (Figure 2). Further fractionation of the methanol extract was carried out using n-hexane.

Some of the compounds in red betel leaf methanolic extract were extracted in the soluble and insoluble fractions of n-hexane, but 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.

Caffeic acid, p-coumaric acid, cyanidin 3-O-glucoside, tannin, and gallic acid are identified to be responsible 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 α-amylase and α-glucosidase enzyme inhibitor [13, 15]. Petroleum ether and methanolic extracts from tropical almond are rich in tannins that had antidiabetic activity [16]. Chemically tannins are complex substances, they usually occur mixtures of polyphenol that difficult to separate because they do not crystallize.

According to Barret *et al.*, [15], larger and more complex tannins, ore effectively inhibited the enzymes. In addition to tannins, other groups of compounds showed enzyme inhibitory activity. The ethyl acetate fraction of red betel leaf extract contain the terpenoid (Columbine) which was stronger in inhibiting the activity of the α-glucosidase enzyme than other compounds [9]. The active substance in the extract responsible for α-glucosidase inhibition is affected by many factors such as harvest time, cultivation, storage conditions, processing, climate, and genetic background [10]. To obtain the compound responsible for the inhibitory activity of α-amylase and α-glucosidase enzymes, red betel leaf methanolic extract can be fractionated with non-polar solvents such as n-hexane. Considering the solubility properties and reported activity of tannins, further research is needed on the activity of compounds in the insoluble n-hexane fraction and the soluble n-hexane fraction in inhibiting the activity of α-amylase and α-glucosidase enzymes.

4. CONCLUSIONS

The antidiabetic ingredients of red betel leaf can be extracted using methanol. Red betel leaf methanolic extract has α-amylase and α-glucosidase enzyme inhibitory activity. Fractionation using n-hexane as solvent was effective in separating the compounds in red betel leaf 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 inhibitory activity of α-amylase and α-glucosidase enzymes from red betel leaf methanolic extract.

AUTHORS' CONTRIBUTIONS

Yustina Sri Hartini: Conceived and designed the experiments; analysed and interpreted the data; contributed reagents, material, analysis tools or data; performed the experiments; wrote the paper.

Dewi Setyaningsih: Contributed reagents, performed the experiment; analysed and interpreted the data.

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