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RESEARCH ARTICLE

α-Glucosidase inhibitory activities of *Loranthus ferrugineus* and *Peperomia pellucida* extracts

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Abstract

Introduction: The usage of traditional medicines in Indonesia has recently increased following the rise in regional COVID-19 cases. They were used to alleviate the symptoms of patients with pre-existing condition such as heart diseases, diabetes mellitus, and inflammation. They were also used to boost peoples immune system. *Loranthus ferrugineus* (Loranthaceae) and *Peperomia pellucida* (Piperaceae) were two medicinal plants used as anti-diabetes drugs. **Aim**: This study aimed to measure the anti-diabetic activity of extracts of *L. ferrugineus* branches and the aerial parts of *P. pellucida*. **Method**: Dried branches of *L. ferrugineus* were macerated using *n*-hexane, ethyl acetate, and methanol, respectively. Fresh aerial parts of *P. pellucida* were macerated with methanol and reduced the volume *in vacuo.* The extract was fractionated with *n*-hexane and ethyl acetate. These extracts and its fractions were dried and tested *in vitro* with α-glucosidase. **Results**: α-Glucosidase inhibitory activities of ethyl acetate and methanol of *L. ferrugineus* were 89.21% and 92.98% at 500 ppm. However, there was no significant activity detected for the *n*-hexane extract. α-Glucosidase inhibitory activities of methanol extract, and its *n*-hexane and ethyl acetate fractions of *P. pellucida* were 8.26%, 0.83% and 28.19% at 500 ppm. **Conclusion:** The methanol extract of *L. ferrugineus* and the ethyl acetate extract of *L. ferrugineus* are considered to have potential to be developed further as therapeutics.

Introduction

The use of plants in traditional medicines has always been of public interest because of their usually negligible side effects. Plants are rich sources of secondary metabolites, which can be used for therapeutic purposes, including treating diabetes mellitus (DM). The greater the plants' diversity in both number and origin, the richer the secondary metabolites they produce in mixtures.

In the past two years, the use of traditional medicines (TM) has increased significantly as the number of COVID-19 cases increased. The purpose of using TM is to prevent infections of the SARS-COV-2 virus and alleviate co-morbidity symptoms in patients with heart disease, inflammatory and/or DM.

DM is the fifth deadliest disease in the world and was responsible for causing 1.5 million deaths worldwide in 2012. In 2014 it was reported that 8.5% of the global adult population were suffering from DM (World Health Organisation, 2016). The World Health Organisation (WHO) reported that the number of people suffering from DM would reach 642 million by the year 2040, the majority from developing countries in Asia. Globally, the majority of DM cases are categorised as being Type 2 diabetes mellitus (T2DM) (Mustafa *et al.*, 2016). This disease is a serious metabolism disorder common in modern society. This disease is marked by the increase of glucose level in the blood (hyperglycemia), caused by insulin resistance or lack of insulin production by β-pancreatic cells.

Two plant species that are commonly used for the treatment of DM are *benalu kopi* (*Loranthus ferrugineus*) (Ameer *et al.*, 2015) and *sirih Cina* (*Peperomia pellucida*) (Susilawati *et al.*, 2017). *L. ferrugineus* was reported to possess quercetin,

quercetin 3-O-α-L-rhamnopyranoside and some phytosterols. *Peperomia pellucida* was known to contain 1,4-bis (benzo [d] [1,3-dioxol-5-yl) hexahydrofural [3,4-c] furan, (4R)-4-(bis(7-methoxybenzo[d] [1,3] dioxol-5-yl) methyl)-3-methylhydrofuran-2(3H)-one and some other peperomin group of compounds.

Based on these facts, α-glucosidase inhibition of extracts and fractions from these plants was examined. The results from this study aim to contribute to the discussion on developing herbal medicine or for the standardisation of herbal biological activities.

Method

Collection of plant materials

Branches of *L. ferrugineus* (Figure 1) were collected in Pekanbaru, Riau Province, Indonesia. Aerial parts of *P. pellucida* (Figure 2) were collected in Pekanbaru. Voucher specimens were identified by Professor Fitmawati from the Department of Biology, Faculty Mathematics and Natural Science, Riau University, Indonesia.

Figure 1: *Loranthus ferrugineus* (Loranthaceae)

Figure 2: *Peperomia pellucida* (Piperaceae)

General experimental procedure

The absorbance of the enzyme assay reaction mixture was measured by TriStar LB 941 multimode microplate reader (Berthold Technologies) at 405 nm.

Sample preparation

100g of *L. ferrugineus* branches were air-dried prior to macerating using *n*-hexane, ethyl acetate, and methanol, respectively. The maceration processes were performed three times for 24 hours with 30-minute ultrasonication. These extracts were dried *in vacuo* to afford 0.39g of *n*-hexane extract, 2.19g of ethyl acetate extract and 5.50g of methanol extract. The samples were labelled as LF-H for the *n*-hexane extract, LF-E for the ethyl acetate extract and LF-M for the methanol extract. 100g of *P. pellucida* fresh aerial parts were macerated with methanol. The volume of this extract was then reduced *in vacuo*. This methanol extract was then fractionated with *n*-hexane (3 x 50mL) followed by ethyl acetate (3 x 50mL). All fractions and the remaining methanol extract were dried *in vacuo*. The *n*-hexane extract (13.50g), ethyl acetate extract (7.00g), and methanol extract (5.00g) were labelled as PP-H, PP-E and PP-M, respectively.

In vitro α-glucosidase inhibition assay

The α-glucosidase inhibitory activity was assessed by the standard method (Sheng *et al*., 2014) with some modifications. The enzyme solution was prepared by dissolving 1mg of α-glucosidase in 100mL of phosphate buffer (pH 7), which contained 200mg of bovine serum albumin. Before being used, 1 mL of enzyme solution was diluted 25 times with phosphate buffer (pH 7). The reaction mixture was prepared in the microplate wells, which consisted of 25µl of 20mM *p*-nitrophenyl-*D*-glucopyranose as substrate and 50µl of 100mM phosphate buffer (pH 7). Each extract was dissolved in DMSO, and aliquots of the sample (10µL) were added to the reaction mixture to get a final concentration of 500µg/mL. Solution of 1% acarbose (Glucobay) was prepared with phosphate buffer pH 7. Then it was mixed with 2N HCl of equal volume (1:1) and was centrifuged. Aliquots of supernatant (10µL) were taken and added into the reaction mixtures at a final concentration of 0.5 µg/mL. Blanks, controls and each concentration of samples were done in triplicate. Following incubation at 37°C for five minutes, 25µl of enzyme solution was added into the reaction mixture and incubated for a further 15 minutes. The enzyme reaction was stopped by adding 100µl of 0.1M Na_2CO_3. Blanks, controls, and samples absorbance of the *p*-nitrophenol product were measured by microplate reader spectrophotometer at 405 nm wavelength.

IC_{50} values were recorded for samples that showed very high inhibition percentages (LF-E and LF-M). The measurement procedure was conducted in the same method. The concentration of samples was set to 31.25, 62.50, 125.00, 250.00 and 500.00 ppm. Probit analysis was employed to get the IC_{50} values.

Results

α-Glucosidase inhibitory activity was tested on LF-H, LF-E, LF-M, PP-H, PP-E and PP-M. The inhibitory test results at 500 ppm are shown in Figure 3. LF-E and LF-M showed activities of more than 90% at 500 ppm. Therefore, IC_{50} values of these extracts were measured. The IC_{50} values of LF-E and LF-M were 184.69 and 157.22μg/mL, respectively.

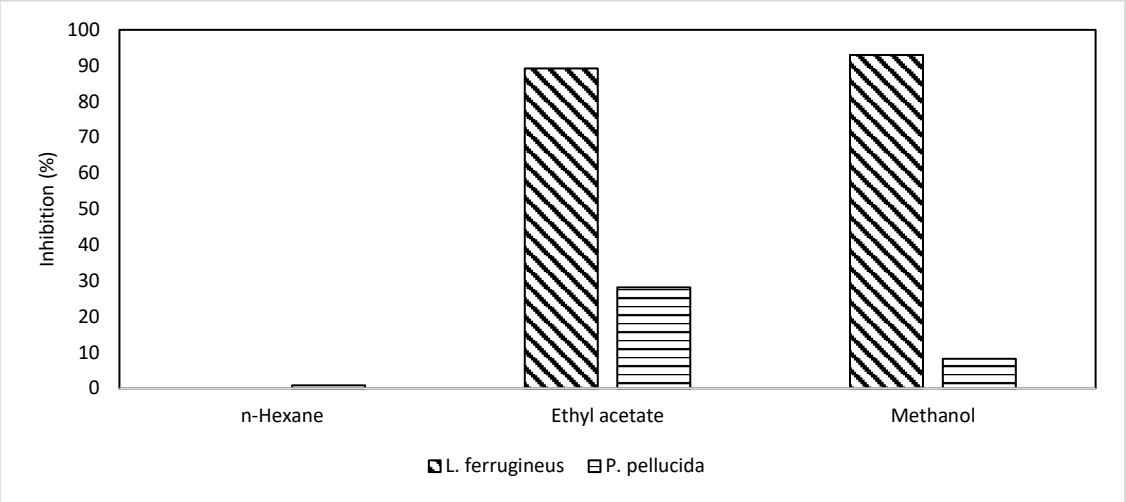

Figure 3: α-Glucosidase inhibitory activities of *L. ferrugineus* and *P. pellucida* extracts at 500 ppm from three different solvents

Discussion

The branches of the plants were used in this study because they possessed a high concentration of antioxidants. The branches of *L. ferrugineus* were also easy to dry. Therefore the maceration process was started with *n*-hexane followed by ethyl acetate and methanol, respectively. The highest concentration of extract produced was obtained with the methanol extract (5.50g), followed by ethyl acetate extract (2.19g) and *n*-hexane extract (0.39g).

The aerial parts of *P. pellucida* were isolated as fresh material since these samples contained a high portion of water. During the air-drying process, they tended to brown (become oxidised). Therefore, the maceration procedure was started with methanol. This methanolic extract was then partitioned with n-hexane and ethyl acetate to afford non-polar and semi-polar fractions, respectively. The amount of extract collected was 13.50g for *n*-hexane extract, 7.00g for ethyl acetate extract, and 5.00g for methanol extract.

α-Glucosidase inhibitory activities of *L. ferrugineus* extract at 500 ppm were 89.21% for ethyl acetate extract (LF-E) and 92.98% for methanol extract (LF-M). However, there was no activity for *n*-hexane extract (LF-H). Meanwhile, α-glucosidase inhibitory activities of *P. pellucida* extracts at 500ppm were 0.83% for *n*-hexane extract (PP-H), 28.19% for ethyl acetate extract (PP-E) and 8.26% for methanol extract (PP-M). Acarbose was used as a positive control with an inhibition value of 99.94%. Since LF-E and LF-M showed activities of more than 90% at 500ppm, IC50 values were measured. The IC50 values of LF-E and LF-M were 184.69 and 157.22 μg/mL, respectively.

Conclusion

α-Glucosidase inhibitory activities from high to low are methanol extract of *L. ferrugineus* (LF-M), ethyl acetate extract of *L. ferrugineus* (LF-E), ethyl acetate extract of *P. pellucida* (PP-E), methanol extract of *P. pellucida* (PP-M) and *n*-hexane extract of *P. pellucida* (PP-H). There is no activity for *n*-hexane extract of *L. ferrugineus* (LF-H). Based on the IC_{50} values, the methanol extract of *L. ferrugineus* (LF-M) and the ethyl acetate extract of *L. ferrugineus* (LF-E) are considered to have the potential to be developed further into therapeutics.

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