Screening for potential antidiabetes and antioxidant activities of selected plants from East Kalimantan, Indonesia

RICO RAMADHAN[1,♥], ALFINDA NOVI KRISTANTI[1], RUDIANTO AMIRTA[2], IRAWAN WIJAYA KUSUMA[2], PREECHA PHUWAPRAISIRISAN[3], MUHAMMAD TAUFIQ HAQIQI[2,4,5], SAPARWADI[6]

[1]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga. Campus C, Mulyorejo, Surabaya 60115, East Java, Indonesia.
Tel./fax.: +62-31-5936501, ♥email: rico.ramadhan@fst.unair.ac.id
[2]Faculty of Forestry, Universitas Mulawarman. Samarinda 75119, East Kalimantan, Indonesia
[3]Center of Excellent in Natural Products, Department of Chemistry, Faculty of Science, Chulalongkorn University. Bangkok 10330, Thailand
[4]Plant Biomass Utilization Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University. Bangkok 10330, Thailand
[5]Program in Biotechnology, Faculty of Science, Chulalongkorn University. Bangkok 10330, Thailand
[6]Yayasan Biosfer Manusia (BIOMA). Jl. A Wahab Syaranie, Sempaja, Samarinda 75119, East Kalimantan, Indonesia

Abstract. *Ramadhan R, Kristanti AN, Amirta R, Kusuma IW, Phuwapraisirisan P, Haqiqi MT, Saparwadi. 2019. Screening for potential antidiabetes and antioxidant activities of selected plants from East Kalimantan, Indonesia. Biodiversitas 20: 1820-1826.* Ten selected plants in this study have been used traditionally in West Kutai, East Kalimantan as medicinal plants. This study aimed to screen and evaluate the in vitro antidiabetic and antioxidant activity of methanol extracts of selected plants from East Kalimantan. The antidiabetic activity was carried out by α-glucosidase inhibitory activity with maltose and sucrose as substrates while antioxidant activity was determined by free radicals scavenging activity against DPPH, ABTS and Nitric Oxide (NO). The results showed that *Garcinia riedeliana* extract had strong inhibitory activity with an IC$_{50}$ value of 0.04 mg/mL followed by *Shorea laevis*, *Pternandra azurea* and *Bridelia tomentosa* with IC$_{50}$ values of 0.12; 0.15 and 0.16 mg/mL in maltose respectively, while quercetin as positive control had an IC$_{50}$ value of 0.08 mg/mL. *G.riedeliana* extract also showed good α-glucosidase inhibitory activity with an IC$_{50}$ value of 0.23 mg/mL in sucrose as substrate. The results on antioxidant activity showed that all extracts have IC$_{50}$ value against free radicals DPPH, ABTS, and nitric oxide. The most active extract as α-glucosidase inhibitory activity and antioxidant activity was *G. riedeliana* extract. These findings provide the scientific evidence that one species of medicinal plants from East Kalimantan might be potential as an antioxidant and α-glucosidase inhibitor.

Keywords: Anti-diabetic, antioxidant, East Kalimantan, free radical scavenging, α-glucosidase

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by prolonged hyperglycemia (Liu et al. 2009). In the year 2000, there were 171 million people with DM, and the number was increasing year to year (Wild et al. 2004). DM is a metabolic disorder caused by several factors such as a reduction of insulin secretion in the pancreas and opposed to insulin action in different tissues in the body, causing a decrease in sugar absorption (Hwang et al. 2011). Postprandial hyperglycemia (PPHG) is the main factor causing diabetes mellitus (DM) and its complication with multiple factors mechanisms (Labonté et al. 2006). Oxidative stress is responsible for the pathogenesis process of diabetes and its complications. Therefore, the search for an antidiabetic agent which also able to overcome oxidative stress would be potential to treat diabetes. At present, the treatment of diabetic patients focuses on controlling PPHG by using analogs targets such as α-glucosidase and α-amylase (Bischoff 1994). Acarbose, miglitol, and voglibose are famous synthetic inhibitors of α-glucosidase that most used for controlling PPHG (Bischoff 1994; Johnston et al. 1998). However, these inhibitors cause side effects such as flatulence, vomiting,

and diarrhea, so consumption used these inhibitors should be limited (Hanefeld 1998; Chakrabarti and Rajagopalan 2002). Several studies on natural products for evaluating free radical scavenger activity and inhibitor α-glucosidase with low side effects have been carried out (Benalla et al. 2010). On the other hand, previous research by Vadivel et al. (2012) reported that natural products with free radical scavenging activities have the potential to reduce the effects of oxidative damage and prevent diabetes complications. Therefore, searching the natural product with the potential to prevent oxidative stress and overcome various diabetic disorders and their complications is more beneficial.

Many medicinal plants have been used to treat diabetes in traditional healthcare systems. On the other hand, a study on potential plants as antidiabetes and antioxidant from East Kalimantan are still very limited. Information about the use of plants from generation to generation for religious purpose and herbal medicine from the East Kalimantan local community (Dayak community) is very important (Joshi et al. 2004). Therefore, in this study, we evaluated ten selected plants from East Kalimantan to determine their activity as free radical scavengers and the inhibitor of α-glucosidase. It was done to provide the

scientific background of the use of plants as herbal medicine. To the best of our knowledge, this study will be the first report on the α-glucosidase activity as well as free radical scavenging activity of selected plants from East Kalimantan.

MATERIALS AND METHODS

Plant collection and identification

Selected plant species used in this study were collected from forest sites of West Kutai District, East Kalimantan Province, Indonesia during the dry season (August 2017). The plant collections, local names, botanical family, part used, voucher specimens number for this study are listed in Table 1. After plant identification, plant voucher specimens were deposited in the Laboratory of Forest Products Chemistry, Faculty of Forestry, Mulawarman University, Samarinda, Indonesia.

Chemical reagents

The chemical used were DMSO (dimethyl sulfoxide) purchased from Merck (Darmstadt, Germany), rat intestinal acetone powder (Sigma Aldrich), and ABTS (2,2`-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) purchased from Sigma Chemical Co (St. Louis, MO, USA) and DPPH (1,1-diphenyl-2-picrylhydrazyl), ascorbic acid, sulphanilic acid, naphthyl ethylenediamine dihydrochloride, sodium nitroprusside and potassium perchlorate were of analytical grade.

Plant extraction

Twig, stems and branch of selected plants were washed with distilled water and dried at room temperature, cut in small pieces and ground into powder. The dried powder from each plant species was weighed to approximately 100 g and extracted by maceration method at room temperature using 400 mL methanol thrice with mild shaking. The filtrate was filtered through filter paper (Whatman Grade 42). The filtrate was concentrated under vacuum in a rotary evaporator (R100, BUCHI) to obtain concentrated methanol extracts. The methanol extracts were stored at-20°C for further use.

Antidiabetic activity

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of the methanol extracts was assayed according to the procedure described previously by Shobana et al. (2009) with slight modifications. Briefly, rat intestinal α-glucosidase powder (Sigma Aldrich) was dissolved in buffer saline as crude enzyme solution, sources of maltase and sucrase. Extracts were individually dissolved in dimethylsulfoxide (DMSO) with various concentrations (0.0025-0.3125 mg/mL), 10 μL of the extract from each concentration was added with phosphate buffer (pH 6.9, 0.1 M, 30 μL). The reaction was initiated by adding 20 μL of a substrate solution (10 mM maltose or 100 mM sucrose, followed by adding crude enzyme solution (20 μL) and glucose kit (80 μL) respectively. The reaction mixture was incubated at 37°C for 10 min (maltose) and 40 min (sucrose), respectively. The amount of glucose released from reaction mixtures was quantified using a microplate reader (TECAN 50) in 96 microwell plate at 503 nm. Quercetin was used as a positive control from natural products. The percentage of inhibition (%) was calculated as follows:

% Inhibition = (Abs control-Abs sample)/ Abs control x 100.

The concentration of extract which inhibited 50% α-glucosidase activity under reaction conditions was defined as IC$_{50}$ value.

Antioxidant activity

DPPH radical inhibitory activity

DPPH radical scavenging activity of selected plant extracts was measured according to the method by Ramadhan and Phuwapraisirisan (2017). Twenty μL of an extract with serial concentrations (0.04-1 mg/mL) was added to 80 μL of 0.01 M DPPH in methanol. The mixture was homogenized and stored in the dark for 20 min. After incubation, the absorbance of the mixture was measured using a microplate reader (TECAN 50) in 96 microwell plate at 517 nm. Ascorbic acid was used as a positive control. The percentage of free radical scavenging activity was calculated using the equations as follows:

% scavenging activity = (Abs control-Abs sample)/ Abs control x 100

Table 1. Selected plants collected from East Kalimantan, Indonesia plants for antidiabetic and antioxidant evaluation

Plant species	Local names	Botanical family	Part used
Garcinia riedeliana	Kayu duling	Clusiaceae	Stem
Shorea laevis	Abanyit	Dipterocarpaceae	Stem
Tristaniopsis whiteana	Pelawan	Myrtaceae	Stem
Endiandra ochracea	Palan bayi	Lauraceae	Stem
Bridelia tomentosa	Serapak lungun	Phyllanthaceae	Branch
Shorea ovalis	Awang buaq	Dipterocarpaceae	Stem
Gmelina arborea	Jati Putih	Lamiaceae	Stem
Macaranga gigantea	Serkong	Euphorbiaceae	Stem
Licania splendens	Kacang	Chrysobalanaceae	Stem
Pternandra azurea	Mutun	Melastomataceae	Twig

The lower the absorbance indicates, the higher the free radical scavenging activity. The IC_{50} represent the concentrations of extract required to inhibit 50% of free radical DPPH. According to Miryanti et al. (2011) states that antioxidant activity is considered extremely high if the value of IC_{50} is less than 0.05 mg/mL, high if the value of IC_{50} is between 0.05-0.1 mg/mL, moderate if the value is between 0.1-0.15 mg/mL and low if the value is between 0.151-0.2 mg/mL.

ABTS radical inhibitory activity

This assay was measured using 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method as described by Floegel et al. (2011). The working solution of ABTS was prepared by a combined equal volume of 7 mM ABTS solution and 2 mM potassium persulfate solution followed by incubation process for 24 hours at room temperature in dark condition. Free radical ABTS scavenging activity was assessed by mixing 20 µL extract (0.04-1 mg/mL) with 100 µL of ABTS working solution. The mixture was incubated at room temperature for 60 min. After incubation, absorbance was determined using as a microplate reader (TECAN 50) in 96 microwell plate at 750 nm. The scavenging activity was calculated using a similar formula as follows :

% scavenging activity = (Abs control-Abs sample)/ Abs control x 100.

Nitric oxide radical inhibitory activity

Nitric oxide was generated from sodium nitroprusside in aqueous solution at physiological pH, which interacts with oxygen to produce nitric ions, which was determined using the Griess reactions (Boora et al. 2014). Twenty µL of various concentrations of extracts were added with 30 µL of 5 mM sodium nitroprusside. After incubation for 1 h at room temperature, 100 µL Griess reagent was added in the mixture followed by second incubation for 10 min. Vitamin C was used as a standard.

Pink chromophore was measured spectrophotometrically at 540 nm. Scavenging activity of Nitric oxide (%) was calculated using the following formula as in DPPH inhibitory activity as follows:

% scavenging activity = (Abs control-Abs sample)/ Abs control x 100.

Data analysis

All data are given as the mean±SD of three measurements and analyzed by nonlinear regression analysis. Quantitative data obtained were analyzed descriptively. All measurement was done in triplicate.

RESULTS AND DISCUSSION

Antidiabetic activity of selected plant extracts from East Kalimantan

This study was conducted to determine the antidiabetic potential of extracts of selected plants from East Kalimantan using the α-glucosidase method, measured using the TECAN 50 Infinite microplate reader. The α-glucosidase test was carried out for initial screening to ensure that the extract of the selected plants has α-glucosidase inhibitory activity. Enzymes of α-glucosidase in the brush of small intestinal cells have a crucial role in the digestion of carbohydrates in human metabolism, including maltase and sucrase. One method to treat diabetes mellitus is by inhibiting carbohydrate digestive enzyme activities such as α-glucosidase to reduce blood glucose levels (Bharatham et al. 2008; Akhter et al. 2013). The principle this assay is to retard the absorption of glucose by inhibiting of carbohydrate-hydrolyzing enzymes at the terminal of non-reducing 1-4 linked (Chiba 1997; Bhandari et al. 2008).

Table 1. The IC_{50} values of the α-glucosidase inhibitory effect of selected plants

Samples	IC$_{50}$ (mg/mL) Substrates	
	Maltose	**Sucrose**
Garcinia riedeliana	0.09 ± 0.02	0.23 ± 0.01
Shorea laevis	0.12 ± 0.02	53.83±0.59
Tristaniopsis whiteana	1.96 ± 0.77	NI[b]
Endiandra ochracea	0.44 ± 0.19	NI
Bridelia tomentosa	0.16 ± 0.01	4.09 ± 1.80
Shorea ovalis	1.31 ± 0.52	NI
Gmelina arborea	0.22 ± 0.04	78.30 ±1.60
Macaranga gigantea	0.26 ± 0.02	NI
Licania splendens	0.21 ± 0.01	114.05±13.37
Pternandra azurea	0.15 ± 0.03	4.60 ± 0.02
Quercetin[a]	0.083 ± 0.01	0.034 ± 0.01

Note: [a] Positive control; [b] No inhibition, inhibitory activity was less than 30% at 0.3125 mg/mL (final concentration); Each value represents the mean ± S.D (n=3)

Table 2. Antioxidant activity of selected plants against free radicals DPPH, ABTS, and Nitric Oxide

Samples	IC$_{50}$ (mg/mL)		
	DPPH	**ABTS**	**Nitric Oxide**
Garcinia riedeliana	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
Shorea laevis	0.58 ± 0.03	0.37 ± 0.01	0.09 ± 0.08
Tristaniopsis whiteana	0.09 ± 0.01	0.05 ± 0.01	0.05 ± 0.03
Endiandra ochracea	0.36 ± 0.01	0.10 ± 0.02	57.83 ± 5.32
Bridelia tomentosa	0.15 ± 0.01	0.18 ± 0.01	0.14 ± 0.05
Shorea ovalis	0.26 ± 0.01	0.32 ± 0.19	0.21 ± 0.09
Gmelina arborea	0.69 ± 0.05	0.72 ± 0.15	NI[b]
Macaranga gigantea	0.25 ± 0.02	0.07 ± 0.04	4.82 ± 0.21
Licania splendens	0.05 ± 0.02	0.07 ± 0.03	0.58 ± 0.25
Pternandra azurea	0.10 ± 0.04	0.67 ± 0.09	0.32 ± 0.03
Ascorbic acid[a]	0.03 ± 0.06	0.12 ± 0.09	0.04 ± 0.01

Note: [a] Positive control; [b] No inhibition, inhibitory effects less than 30% at 1 mg/mL; Each value represents the mean ± S.D (n=3)
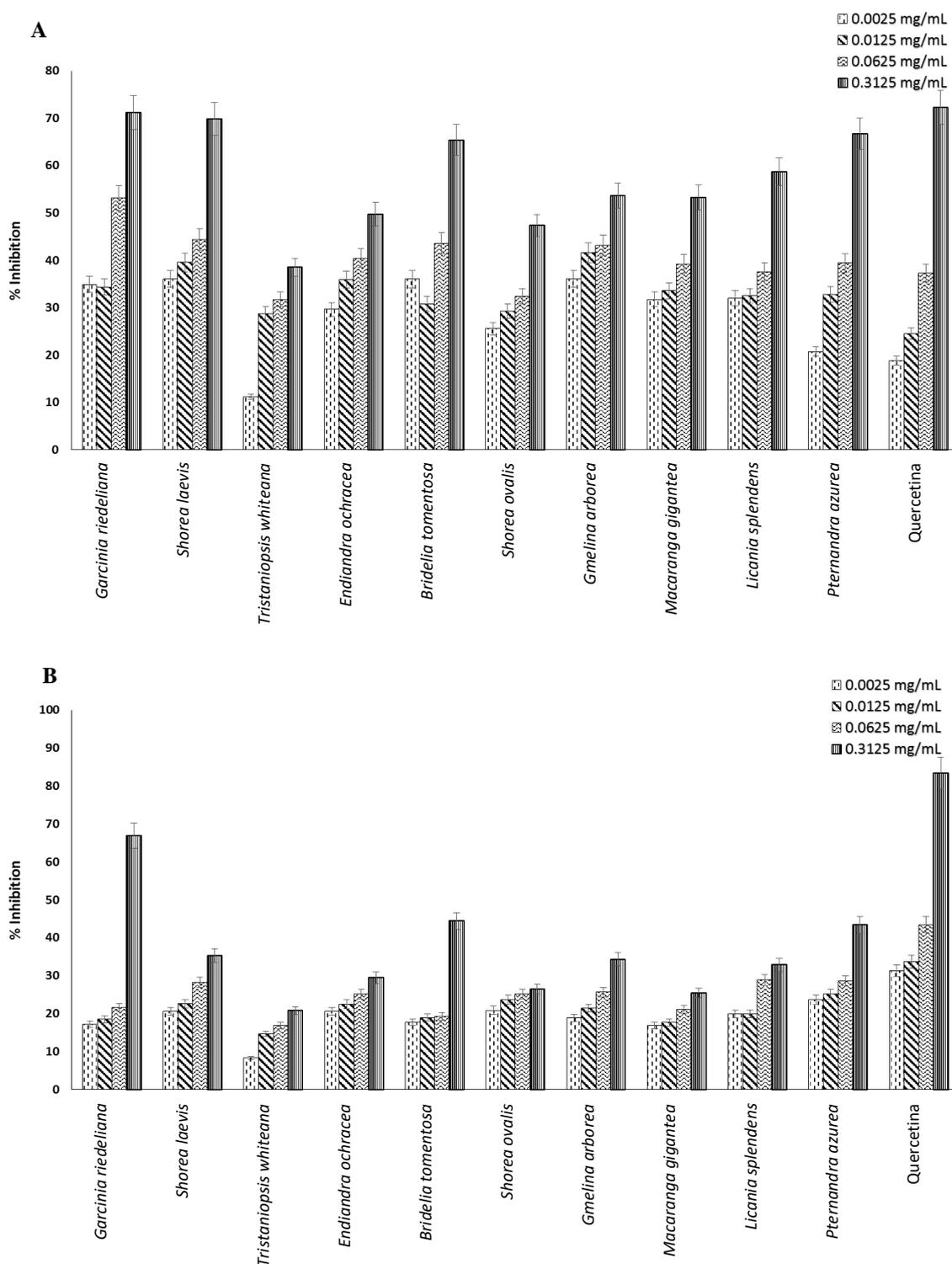
A

B

Figure 1. α-Glucosidase inhibitory activity of ten selected plant extracts with maltose (A) and sucrose (B) as substrates

Results of α-glucosidase (maltose and sucrose as substrates) inhibitory activity of ten selected plant extracts presented in Table 1. Extract of *Garcinia riedeliana* with maltose as a substrate has the strongest α-glucosidase inhibitory activity among the ten extracts with the IC_{50} value of 0.09 ± 0.02 mg/mL that is comparable to IC_{50} value of Quercetin (as positive control) (0.083 ± 0.01

mg/mL). *Garcinia riedeliana* extract have α-glucosidase inhibitory activity with maltose as a substrate 18.8 %, 24.4%, 37.2% and 72.2 % at final concentrations of 0.0025; 0.0125; 0.0625 and 0.3125 mg/mL respectively. According to Kalita et al. (2018) and Rasouli et al. (2017), genus *Garcinia* contains several polyphenolic compounds such as benzophenones, xanthones, coumarins, and flavonoids that

might be responsible for the inhibition of α-glucosidase by several available hydrogen bonds (H-bond) donor and acceptor and the presence of OH groups. Figure 1a showed the percentage of α-glucosidase inhibitory activity of extracts in different concentrations against with maltose as substrate. Fig. 1 showed that *Shorea laevis*; *Bridelia tomentosa*; *Gmelina arborea*; *Macaranga gigantea*; *Licania splendens* and *Pternandra azurea* extracts had better anti-diabetic activity, exhibited more than 50% inhibition with maltose as substrate at the same concentrations. According to Li et al. (2009), quercetin is used as a positive control because it is a natural product that is stronger in controlling blood glucose levels than synthetic inhibitors. Table 1 showed IC_{50} values as inhibitory activity of alpha-glucosidase from selected plant extracts in maltose in the following order: : *Tristaniopsis whiteana* (1.96 ± 0.77 mg/mL) > *Shorea ovalis* (1.31 ± 0.52 mg/mL) > *Endiandra ochracea* (0.44 ± 0.19 mg/mL) > *Macaranga gigantea* (0.26 ± 0.02 mg/mL) > *Gmelina arborea* (0.22 ± 0.04 mg/mL) > *Licania splendens* (0.21 ± 0.01 mg/mL) > *Bridelia tomentosa* (0.16 ± 0.01 mg/mL) > *Pternandra azurea* (0.15 ± 0.03 mg/mL) > *Shorea laevis* (0.12 ± 0.02 mg/mL). The lower the IC_{50} values, the better the anti-diabetic of plant extracts.

The result of α-glucosidase inhibitory activity with sucrose as substrate showed that extract *G. riedeliana* has higher inhibition at various concentrations (0.0025-0.3125 mg/mL) than other extracts (Fig. 1b). As shown in Figure 1b, Extract *T. whiteana*, *E.ochracea*, *S. ovalis*, and *M. gigantea* have inhibition of the α-glucosidase activity of less than 30%, therefore considered as inactive in inhibiting α-glucosidase. The stem bark extract of *G. riedeliana* showed the best α-glucosidase inhibitory activity in maltose and sucrose substrates (Table 1), it indicated that *G. riedeliana* extract has the best antidiabetic activities among the extracts of selected plants in this study. However, extracts of *S. laevis*; *B. tomentosa*; *G. arborea*; *M. gigantea*; *L. splendens* and *P. azurea* extracts had α-glucosidase inhibitory activity in maltose as substrate. Based on literature studies, antidiabetes inhibitory activity of *Garcinia lateriflora* leaves against yeast α-glucosidase were reported by Mahayasih et al. (2017) in various fractions such *n*-hexane (IC_{50} 92.33 µg/mL), ethyl acetate (IC_{50} 34.79 µg/mL) and methanol (IC_{50} 31.27 µg/mL), respectively. Phytochemical components of genus *Garcinia* sp. consists of secondary phenolic metabolites including xanthones, biflavonoids, flavonoids, chalcones and phloroglucinols (Thepthong et al. 2017). Kissinger et al. (2016) reported that *Shorea belangeran* bark extract growth in Kerangas forest had antidiabetic inhibitory activity againt α-glucosidase with an IC_{50} value of 0.816 ppm, which phytochemical of this species contains oligostilbenoid (Tukiran et al. 2005). Furthermore, Gunawan-Puteri and Kawabata (2010) reported that new phenolic groups such as macatannins A (IC_{50} 0.80 mM) and macatannins B (IC_{50} 0.55 mM) from leaf of *Macaranga tanarius* showed antidiabetic inhibitory activity against rat intestinal α-glucosidase. However, our finding results were consisted with those of the above-mentioned study about antidiabetic assay but quite differ with source

of α-glucosidase and part of plants used in assay examined. Therefore, as far as we know, there have been no reports of antidiabetic screening by α-glucosidase inhibitory activity (on the substrate of maltose and sucrose) of selected plants from East Kalimantan mentioned above. Further study to isolate secondary compounds responsible for antidiabetic activity from active extracts is in progress.

Antioxidant activity

The free radical scavenging activity of the selected plant extracts was determined by DPPH, ABTS, and Nitric Oxide assays. The results were presented in Table 2. *G. riedeliana*; *L. splendens* and *T. whiteana* extracts had the IC_{50} value of 0.04 ± 0.01; 0.05 ± 0.02 and 0.09 ± 0.01 mg/mL, respectively which were classified as very strong antiradical scavenging activity against DPPH. According to Miryanti et al. (2011) IC_{50} values < 0.05 mg/mL for antioxidant was very strong. These IC_{50} values were similar with an IC_{50} value of a positive control ascorbic acid (0.03 ± 0.06 mg/mL). The IC_{50} value indicates the concentration of extract requires to inhibit 50% DPPH free radicals into a stable molecule that indicated by changing the color of DPPH from purple to yellow (Li et al. 2012). The lower the IC_{50} value, the higher the antioxidant activities. Several studies of antioxidant activity from several species of plants in this study have been reported. Attanayake et al. 2019 reported that stem bark methanol extract of *G. arborea* from Sri Lanka had antioxidant activity (IC_{50} value of 36.89 ± 1.23 µg/mL). Anjum et al. (2013) reported that *B. tomentosa* extract had free radical activity with an IC_{50} value of 7.55 ± 0.27 µg/mL. The result of this study differs from Anjum et al. (2013) alleged due to according Salim et al. (2016) differences in the place of growth of a species affect the precursors of biosynthesis of secondary metabolites that might be effective to their bioactivity.

Mazlan et al. (2013) and Tajuddin and Zain (2010) reported that *M, gigantea* and *S. ovalis* has weak inhibitory activity against free radical DPPH. However, our finding results demonstrated that *G.riedeliana* and *L. splendens* are categorized as very strong antioxidant. Based on IC_{50} value the extracts of *T. whiteana* and *P. azurea* are categorized as strong antioxidant activity, and *B. tomentosa* is categorized as moderate antioxidant activity. Moreover, *S.ovalis* and *M. gigantea* are categorized as weak antioxidant activity based on category IC_{50} values of Miryanti et al. (2011). Meanwhile, according to Molyneux (2004) states that IC_{50} value of 0.2-1 mg/mL was declared less active but still has potential as an antioxidant, so that *E. ochracea, S. laevis, G.arborea* are categorized less active.

The antioxidant activity of plant extract from East Kalimantan also determined by ABTS free radicals. ABTS (2,2´-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid) is one of the free radicals commonly used to assess antioxidant activity, with the principle of electron transfer and involves the reduction of a colored oxidant. ABTS assay is established on the generation of a blue/green $ABTS^{\bullet+}$ that can be reduced by antioxidant; it generated by reacting ABTS salt with potassium persulfate ($K_2S_2O_8$) (Floegel et al. 2011; Rodriguez-Amanya 2010). Table 2 showed that the IC_{50} of ABTS scavenging activity from ten

selected plant extracts in increasing order: *G.riedeliana = T. whiteana < M. gigantea = L. splendens < E. ochracea < B. tomentosa < S. ovalis < S. laevis < P. azurea < G. arborea*. Based on the results, *G.riedeliana, T. whiteana, M. gigantea, L. splendens,* and *E. ochracea* are categorized strong antioxidant activity with IC$_{50}$ values ranging from 0.05-0.10 mg/mL, while *B.tomentosa* revealed moderate antioxidant activity. Furthermore, *S. laevis, S. ovalis, G. arborea,* and *P. azurea* are categorized weak antioxidant activity based on antioxidant activity category of Miryanti et al. (2011). The lower the IC$_{50}$ value of plant extracts against ABTS radical indicates the higher the antioxidant activities. According to Sanseera et al. (2014) reported that methanol extract from *B. retusa* stems has antioxidant activity against ABTS free radical with IC$_{50}$ value of 0.56 mg/mL, these results differ with present study, which might be due to different present of secondary metabolites that responsible for antioxidant activity. Subramanian et al. (2013) reported the antioxidant activity of methanol extract of *Shorea roxburghii* stem bark detected by ABTS method has strong antioxidant activity with IC$_{50}$ value of 0.056 mg/mL, these results have a linear affect of the presence of bioactive phenolic compounds. Other studies, Verma et al. (2009) reported that methanol extract of *Macaranga peltata* stem bark has antioxidant activity with IC$_{50}$ value of 9.77 ppm. However, the recent study demonstrated that some selected plants from East Kalimantan flora have potential as source natural antioxidant. Based on our knowledge, this is the first report free radical scavenging activity against ABTS of selected species collected from East Kalimantan flora. Moreover, to continuously verify the antioxidant activity of selected plants, we determined antioxidant activity against nitric oxide free radicals.

Nitric oxide is engendered from amino acid L-arginine by vascular endothelial cells; it plays an important role in the inflammatory processes. Nitric oxide is categorized as free radicals because of its unpaired electron; it has an adverse effect when react with superoxide radicals to form highly reactive peroxynitrite anion (ONOO$^-$) (Nagmoti et al. 2012; Sunil et al. 2013). The results in Table 2 showed that *G. riedeliana, S. laevis*, and *T. whiteana* have the same inhibitory activity as positive control ascorbic acid against nitric oxide. Extracts of *B.tomentosa*, *P. azurea*, *S. ovalis*, and *L. splendens* showed moderate inhibitory activity, while *M. gigantea*, *E.ochracea,* and *G. arborea* extracts have lower inhibitory activity compared to ascorbic acid and do not have antioxidant activity based on its IC$_{50}$ value (4.82 and 57.83 mg/mL; no inhibition). The extracts of selected plants inhibited nitrite formation by competing with oxygen, which leads to a reduction in nitric oxide production (Razali et al. 2008). Based on the results of antioxidant activities of ten selected plants from East Kalimantan has clearly recognized that some extract plants has a good potential of antioxidant activity achieved by free radical scavenging against DPPH, ABTS and nitric oxide. Of test extract plants, *G. riedeliana* and *T. whiteana* showed strikingly strong antioxidant activity in all bioassay examined, while *L. splendens* showed strong antioxidant activity in both free radicals against DPPH and ABTS.

Furthermore, extracts of *B.tomentosa* was also categorized moderate antioxidant activity in all bioassay examined.

In summary, to the best our knowledge, this is the first report showed that selected plants from East Kalimantan have anti-diabetic activity based on α-glucosidase inhibitory activity in maltose and sucrose as substrates and antioxidant activities as free radicals scavenger against DPPH, ABTS, and nitric oxide. This study provides scientific evidence of the use of plants in the traditional healthcare system. Furthermore, extracts with good activity as an α-glucosidase inhibitor and antioxidant neede to be studied further to isolate secondary metabolites that may be responsible for antidiabetes and its complications caused by free radicals.

REFERENCES

Akhter F, Hashim A, Khan MS, Ahmad S, Iqbal D, Srivastava AK, Siddiqui MH. 2013. Antioxidant, α-amylase inhibitory and oxidative DNA damage protective property of *Boerhavia diffusa* (Linn.) root. S Afr J Bot 88: 265-272.

Anjum A, Sikder MA, Haque MR, Hasan CM, Rashid MA. 2013. In vitro antioxidant and thrombolytic activities of *Bridelia* species growing in Bangladesh. J Sci Res 5: 343-351.

Attanayake AP, Jayatilaka KAPW, Pathirana C, Mudduwa LKB. 2019. Antioxidant activity of *Gmelina arborea* Roxb. (Verbenaceae) bark extract: *In vivo* and in vitro study. J Med Nut Nutraceut 4: 32-38.

Benalla W, Bellahcen S, Bnouham M. 2010. Antidiabetic medicinal plants as a source of alpha-glucosidase inhibitors. Curr Diabetes Rev 6: 247-254.

Bhandari MR, Jong-Anurakkun N, Hong Gao, Kawabata J. 2008. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata* Haw). Food Chem 106: 247-252.

Bharatham K, Bharatham N, Park KH, Lee KW. 2008. Binding mode analyses and pharmacophore model development for sulfonamide chalcone derivatives, a new class of α-glucosidase inhibitors. J Mol Graph Model. 26: 1202-1212.

Bischoff H. 1994. Pharmacology of alpha-glucosidase inhibition. Eur J Clin Invest 24: 3-10.

Boora F, Chirisa E, Mukanganyama S. 2014. Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. J Food Process 2014: 1-7.

Chakrabarti R, Rajagopalan R. 2002. Diabetes and insulin resistance associated disorders: Disease and therapy. Curr Sci 83: 1533-1538.

Chiba S. 1997. Molecular mechanism in alpha-glucosidase and glucoamylase. Biosci Biotechnol Biochem 61: 1233-1239.

Floegel A, Kim D, Chung SJ, Koo SI, Chun OK. 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Compos Anal 24: 1043-1048.

Gunawan-Puteri MDPT, Kawabata J. 2010. Novel α-glucosidase inhibitors from *Macaranga tanarius* leaves. Food Chem 123 (2): 384-389.

Hanefeld M. 1998. The role of acarbose in the treatment of non-insulin-dependent diabetes mellitus. J Diabetes Complications 12: 228-237.

Hwang S, Kwak SH, Bhak J, Kang HS, Lee YR, Koo BK, Park KS, Lee HK, Cho YM. 2011. Gene expression pattern in transmitochondrial cytoplasmic hybrid cells harboring type 2 diabetes-associated mitochondrial DNA haplogroups. PLoS One 6 (7): e22116. DOI: 10.1371/journal.pone.0022116.

Johnston PS, Lebovitz HE, Coniff RF, Simonson DC, Raskin P, Munera, CL. 1998. Advantages of α-glucosidase inhibition as monotherapy in elderly type 2 diabetic patients. J Clin Endocrinol Metab 83: 1515-1522.

Joshi L, Wijaya K, Sirait M, Mulyoutami E. 2004. Indigenous systems and ecological knowledge among Dayak people in Kutai Barat, East Kalimantan-a preliminary report. ICRAF Southeast Asia Working Paper, Bogor.

Kalita D, Holm DG, LaBarbera DV, Petrash JM, Jayanty SS. 2018. Inhibition ofα-glucosidase,α-amylase, and aldose reductase by potato polyphenolic compounds. PLoS One 13 (1): e0191025. DOI: 10.1371/journal.pone.0191025.

Kissinger. Yamani A, Pitri RMN. 2016. α-Glucosidase inhibitory activity of *Shorea belangeran* of Kerangas forest. J Med Plants Res 10: 356-361.

Labonté ED, Kirby RJ, Schildmeyer NM, Cannon AM, Huggins KW, Hui DY. 2006. Group 1B phospholipase A2-mediated lysophospholipid absorption directly contributes to postprandial hyperglycemia. Diabetes 55: 935-941

Li YQ, Zhou FC, Gao F, Bian JS, Shan F. 2009. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of α-glucosidase. J Agric Food Chem 57: 11463-11468.

Li X, Lin J, Gao Y, Han W, Chen D. 2012. Antioxidant activity and mechanism of rhizoma *Cimifugae*. Chem Cent J 6: 1-10.

Liu LL, Yi JP, Beyer J, Mayer-Davis EJ, Dolan LM, Dabelea DM, Lawrence JM, Rodriguez BL, Marcovina SM, Waitzfelder BE, Fujimoto WY. 2009. Type 1 and type 2 diabetes in Asian and Pacific Islander U.S Youth. Diabetes Care 32: S133-S140.

Mahayasih PGMW, Elya B, Hanafi M. 2017. Alpha-glucosidase inhibitory activity of *Garcinia lateriflora* Blume leaves. J Appl Pharm Sci 7: 100-104.

Mazlan NA, Mediani A, Abas F, Ahmad S, Shaari K, Khamis S, Lajis NH. 2013. Antioxidant, antityrosinase, anticholinesterase, and nitric oxide inhibition activities of three Malaysian *Macaranga* species. Sci World J 2013: 1-9.

Molyneux P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26: 211-219.

Miryanti A, Sapei L, Budiono K, Indra S. 2011. Extraction of antioxidants from the skin of mangosteen fruit (*Garcinia mangostana* L.). University of Catholic Parahyangan, Bandung. [Indonesian]

Nagmoti DM, Khatri DK, Juvekar PR, Juvekar AR. 2012. Antioxidant activity and free radical scavenging potential of *Pithecellobium dulce* Benth seed extracts. Free Rad Antiox 2: 37-43.

Ramadhan R, Phuwapraisirisan P. 2017. A new 4-arylflavan from pericarps of *Horsfieldia motleyi* displaying dual inhibition against α-glucosidase and free radicals. Nat Prod Res 32: 2676-2682.

Rasouli H, Ghazvini SMBH, Adibi H, Khodarahmi R. 2017. Differential α-amylase/α-glucosidase inhibitory activities on plant-derived phenolic compounds: a virtual screening perspective for the treatment of obesity and diabetes. Food Funct 8: 1942-1945.

Razali N, Razab R, Junit S, Abdul AA. 2008. Radical scavenging and reducing properties of extracts of cashew shoots (*Anacardium occidentale*). Food Chem 111: 38-44.

Rodriguez-Amaya DB. 2010. Quantitative analysis, in vitro assessment of bio-availability and antioxidant activity of food carotenoids-A review. J Food Compos Anal 23: 726-740.

Salim M, Yahya, Sitorus H, Ni'mah T, Marini. 2016. The relation of nutrient soil content to the secondary metabolites production in duku plant (*Lansium domesticum* Corr var duku) and it's larvacide potential. Jurnal Vektor Penyakit 10 (1): 11-18. [Indonesian]

Sanseera D, Liawruangrath B, Pyne SG, Liawruangrath S. Determination of antioxidant and anticancer activities together with total and flavonoid contents of *Cleidion javanicum* Bl. and *Bridelia retusa* (L.) A. Juss. Chiang Mai J Sci 43: 535-546.

Shobana S, Sreerama YN, Malleshi NG. 2009. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. Food Chem 115: 1268-1273.

Subramanian R, Subbramaniyan P, Raj V. 2013. Antioxidant activity of the stem bark of *Shorea roxburghii* and its silver reducing power. Springerplus 2: 1-11.

Sunil C, Duraipandiyan V, Ignacimuthu S, Al-Dhabi NA. 2013. Antioxidant, free radical scavenging and liver protective effects of friedelin isolated from *Azima tetracantha* Lam. leaves. Food Chem 139: 860-865.

Tajuddin NA, Zain WZWM. 2010. The antioxidant studies of Dipterocarpaceae species from UiTM forest reverse, Jengka, Pahang; Proceeding of International Conference on Science and Social Research (CSSR) in Kuala Lumpur, Malaysia, December 5-7, 2010.

Thepthong P, Phongpaicit S, Caroll AR, Voravuthikunchai SP, Mahabusarakam W. 2017. Prenylated xanthones from the stem bark of *Garcinia dulcis*. Phytochem Lett 21: 32-37.

Tukiran, Achmad SA, Hakim EH, Makmur L, Sakai K, Shimizu K, Syah YM. 2005. Oligostilbenoids from *Shorea balangeran*. Biochem Syst Ecol 33: 631-634.

Vadivel V, Cheong JN, Biesalski HK. 2012. Antioxidant and type II diabetes-related enzyme inhibition properties of methanolic extract of an underutilized food legume, *Canavalia ensiformis* (L.) DC: Effect of traditional processing methods. LWT-Food Sci Technol 47: 255-260.

Verma M, Raj PV, Chandrasekhar HR, Rao JV, Udupa N. 2009. Screening of plant *Macaranga peltata* for its antioxidant, antimicrobial and cytotoxicity activity. Proceeding of the 2009 International Conference on Biomedical and Pharmaceutical Engineering (ICBPE 2009), 2-4 December 2009, Singapore.

Wild S, Roglic C, Green A, Sicree R, King H. 2004. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 27: 1047-1053.