黒潮圏科学（Kuroshio Science），９－２，108-122, 2016

Research Paper

Inhibition of α-Glucosidase by Methanol Extracts from Wood Bark of *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* Plants Family in West Kalimantan, Indonesia

Fathul Yusro[1, 2*], Kazuhiro Ohtani[3] and Satoshi Kubota[1]

[1] Division of Human Health and Medical Science, Graduate School of Kuroshio Science, Kochi University, Okoh-cho, Kohasu, Nankoku, Kochi 783-8505, Japan

[2] Faculty of Forestry Tanjungpura University, Pontianak, Indonesia

[3] Division of Human Health and Medical Science, Graduate School of Kuroshio Science, Kochi University, Otsu 200, Monobe, Nankoku, Kochi 783-8502, Japan

Abstract

This is the study to evaluate plants that are trusted by people as anti-diabetic medicine in West Kalimantan Indonesia by examining their ability to inhibit α -glucosidase. The plants species examined are *Dracontomelon dao*, *Mangifera foetida, Mangifera pajang, Pentaspadon motleyi* (Anacardiaceae), *Parkia intermedia, Parkia speciosa, Parkia timoriana* (Fabaceae), *Durio dulcis, Durio kutejensis* (Malvaceae), *Baccaurea angulata* and *Baccaurea costulata* (Phyllanthaceae). Methanol extracts from wood bark of these plants were analyzed in terms of inhibition of two kinds of α-glucosidase enzyme, yeast α-glucosidase (maltase) and rat intestinal α-glucosidase (sucrase). The percentage of methanol extract content of wood barks varied from 2.05 to 21.48%. *P. speciosa, D. dao, D. kutejenis, P. intermedia* and *P. timoriana* had strong inhibitory activity on yeast α-glucosidase with the IC_{50} value of 1.92, 3.24, 3.25, 3.27 and 3.65 μg/ml, respectively. In contrast, *P. motleyi, P. speciosa, P. timoriana*, and *B. costulata* showed lower inhibitory activity on rat intestinal sucrase with the IC_{50} value of 930.87, 789.25, 767.20, and 962.73 μg/ml, respectively.

Keywords: Medicinal plants, anti diabetic, wood bark extraction, yeast and rats intestinal enzymes, Parkia, Durio, Mangifera, Baccaurea, Pentaspadon, Dracontomelon

Introduction

Hyperglycemia or high blood sugar level is one of the criteria of diabetes. It is caused by the disruption of a metabolic system of carbohydrates, proteins and fats resulting in complications of the kidneys, eyes and cardiovascular system (Oyedemi *et al*., 2011, Patel *et al*., 2012, Zhang and Li 2014). The effect of these complications can lead to the death of diabetic patients. Diabetes is the third cause of the death after cancer, cardiovascular and cerebrovascular diseases (Patel *et al*., 2012).

The number of diabetic patients in the world in 2014 were 386.7 million people with the prevalence level is 8. 3%, and this will increase up to 591.9 million people in 2030 (International Diabetes Federation, 2014). Indonesia, that is one of the developing countries, has changed the life style drastically. Especially, high fat diets and less exercises increased the number of diabetic patients. According to Infodatin (2014), the prevalence of diabetic patient above 15 years old in 2013 was 2.1%, increasing two-fold compared with that in 2007 that was the sixth

Received 12 February 2016; Accepted 21 February 2016

*Corresponding author: Fathul Yusro: e-mail: fathulyusro@gmail.com, TEL: 0806288140

cause of death. Diabetic type 2 (non-insulin-dependent diabetes mellitus) is most dominant, almost 90% of all diabetic cases belong to this type in the world (Hasan *et al*., 2014). This condition is very worrying and needs serious treatment to solve this problem.

One of the ways that can be used as a therapeutic approach in the treatment of diabetes is postprandial control of blood sugar levels by delaying of the glucose absorption. α-glucosidase enzymes in the epithelial mucosa of small intestine have a function to break up the complex carbohydrates in the glycoside bonds to make mono and disaccharides such as maltase and sucrase that can be absorbed by intestinal epithelial cells. Inhibition of α-glucosidase in the digestive system will delay the digestion of carbohydrates and extend the digestion time which decreases the rate of glucose absorption resulting in the reduction of blood glucose level. The inhibition of α-glucosidase is therapeutic for patients of diabetic with type 2 (Jaiswal *et al*., 2012).

Currently, modern drugs had been widely available for decreasing blood sugar levels. The use of these drugs should be done intensively. The extensive use of these drugs, however, has side effects such as flatulence, diarrhea, abdominal discomfort, nasopharyngitis, upper respiratory tract infection, headache, allergic, anaphylactoid reactions, angioedema and exfoliate dermatologic reactions (Dicker, 2011, Xu *et al*., 2014, Antu *et al*., 2014, Kashtoh *et al*., 2014). Although it has already been used intensively, sometimes these drugs are unable to control hyperglicemia and eventually the diabetic patient condition would be worried (Ablat *et al*., 2014).

The utilization of traditional herbal medicine to treat diabetes has been accepted by the people who live in the rural area or in the urban/close to modern medical center. Traditional medicine is chosen because it has several advantages such as low side effects and relative safety. Especially, the price is lower for patients who cannot afford to buy medicine at higher price (Ablat *et al*., 2014).

People know about diabetes based on some indication such as high in urine quantities, increase of hungry sensation, a lot of eating and drinking, reduction of weight body, listless, foot numb and requires of long healing time if body injuries (Soenanto, 2005). Based on the results of the previous studies, it is known that there are 22 species of plants that have been traditionally used to treat diabetes by 3 Dayak subethnic tribes, Iban, Kanayant and Ketungau in Kalimantan Barat Province, Indonesia (Yusro *et al*., 2015). Some herbs such as the followings have anti-diabetic compounds. For example, *Garcinia mangostana* has anti-diabetic compound named xanthon (Chaverri *et al*., 2008). *Tinospora crispa* was tested in patients with diabetic type 2 (Klangjareonchai and Roongpisuthipong, 2012) and *Phyllanthus niruri* can decrease blood glucose levels of the model mice (Okoli *et al*., 2009). However, some of other medicinal plants have not yet scientifically proven to have anti-diabetic properties.

The purpose of this study is to analyze the anti-diabetic effects of several plants that are trusted by people in West Kalimantan Indonesia to be efficacious as diabetic medicine. We found several of these plants have inhibitory effects on yeast α-glucosidase (maltase) and rat intestinal sucrase. All of plant species that were examined belong to the *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* plants family (Table 1, Fig. 1). The plant part used is wood bark. The wood bark selected because of their high content of methanol extracts with a variety of chemical compounds that may allegedly have the ability to inhibit α-glucosidase. The source of α-glucosidase enzyme will be used is yeast (EC. 3.2.1.20) from *Saccharomyces cerevisae* containing maltase and rat intestinal acetone powder containing sucrase substrat (rat intestinal sucrase). Different enzyme source and kind of glucose containing in the enzyme will affect to inhibitory α-glucosidase activity (Tadera *et al*., 2006, Jo *et al*., 2010).

Table 1. Medicinal plants species of *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* and their reported activities.

No.	Local Name Scientific Name Family	Traditional Utilization*		Literature Review
		Main Use	Medication	
1	Pelanjau *Pentaspadon motleyi* Anacardiaceae	Fruits: consumption Wood: construction	Exudate : Skin infection	Wood extractive of *P. motleyi* contains phenolic compounds and has anti-fungal activity (Yusro *et al.,* 2010)
2	Asam kemantan *Mangifera foetida* Anacardiaceae	Fruits: consumption Wood: construction	Leaf : Fever	Fruit of *M. foetida* have antioxidant compounds such as flavonoid, carotenoid, and ascorbic (Tyug *et al.,* 2010, Mirfat *et al.,* 2013)
3	Asam bawang *Mangifera pajang* Anacardiaceae	Fruits and skin fruits: consumption, wood: construction	Fruits : Increase of appetite	Phenolic compounds of fruit peel *M. pajang* have the free radical scavenging activity (Hassan *et al.,* 2011) and contain carotenoids that is antioxidant (Khoo *et al.,* 2010)
4	Sengkuang *Dracontomelon dao* Anacardiaceae	Fruits: consumption Wood: construction	Stem : Diarrhea, stomachache	Leaf extract of *D. dao* has antibacterial and antifungal compounds such as tannins, flavonoids, sterol, saponin, and triterpenoids (Khan and Omoloso, 2002)
5	Petai kedaung *Parkia timoriana* Fabaceae	Fruits: consumption	Fruit : Stomachache	All of part plants contain phytosterols (Tisnadjaja, 2005), methanol extracts are hepatoprotective on paracetamol induced liver damage in wistar rats (Ajibola *et al.,* 2013)
6	Petai Pelepah Pendek *Parkia speciosa* Fabaceae	Fruits: consumption	**Roots : Anti-diabetic:, stomachache	Stigmast-4-en-3-one from fruit and pod of *P. spesiosa* as anti-hyperglycemia (Jamaludin and Mohamed, 1993, Jamaludin *et al.,* 1995) and leaf extract for antioxidant and antiulcer (Al Batran *et al.,* 2013)
7	Petai pelepah panjang *Parkia intermedia* Fabaceae	Fruits: consumption	Roots : Anti-diabetic	-
8	Durian meranang *Durio dulcis* Malvaceae	Fruits: consumption Wood: construction	Inner fruits bark : Stomachache	-
9	Durian Pekawai *Durio kutejensis* Malvaceae	Fruits: consumption Wood: construction	Inner fruits bark : Stomachache	Wood bark *D. kutejensis* contains triterpenoid (Rudiyansyah and Garson, 2006), fruit extracts has anti-oxidants properties with potential for hypopigmentation and for use as skin lightening agent (Arung *et al.,* 2015).
10	Enceriak *Baccaurea costulata* Phyllanthaceae	Fruits: consumption	Roots : Anti-diabetic	-
11	Belimbing merah *Baccaurea angulata* Phyllanthaceae	Fruits: consumption	**Roots bark : Anti-diabetic	Fruit of *B. angulata* with phenolic compound that is antioxidant (Jauhari *et al.,* 2013, Ahmed *et al.,* 2014)

* : Based on the interview to local people upon take the sampling (Malay and Java tribes) in Kuala Buayan village, Meliau Distric, Sanggau Regency, West Kalimantan Province, Indonesia

** : Yusro *et al.,* 2015

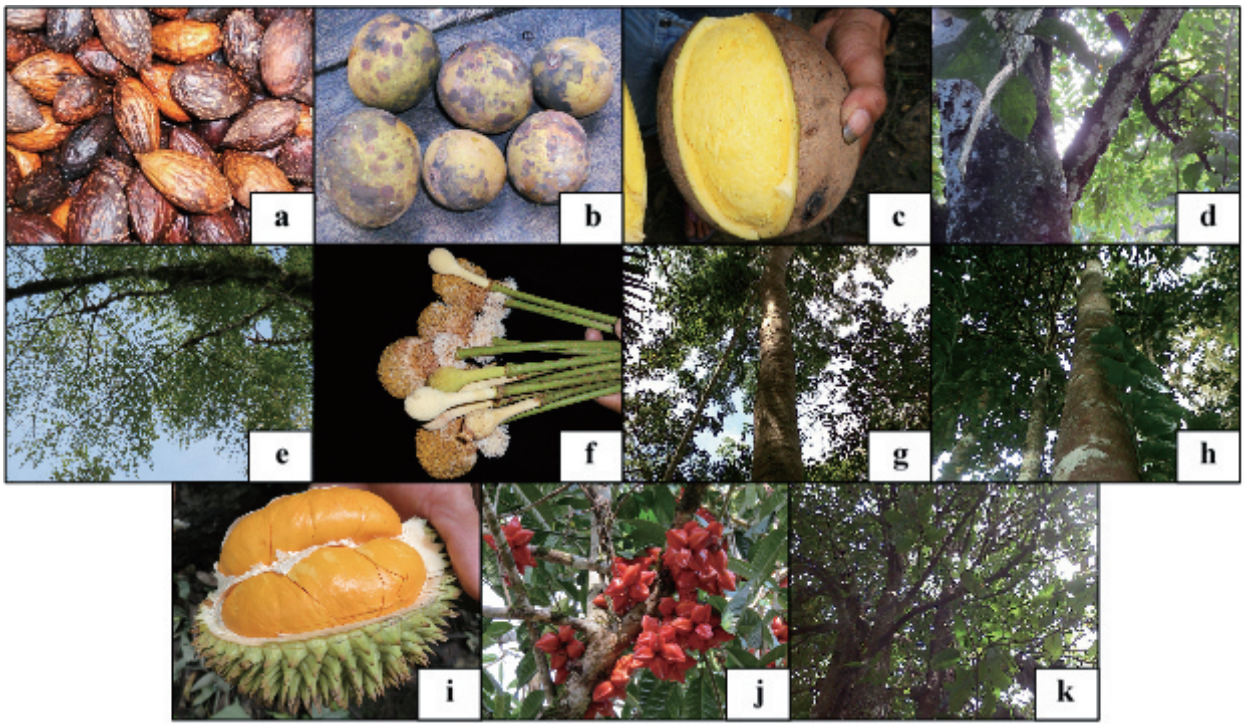

Figure 1. Some of plants part of *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* plants family. a. *P. motleyi* (fruits), b. *M. foetida* (fruits), c. *M. pajang* (fruits), d. *D. dao* (tree), e. *P. intermedia* (tree), f. *P. speciosa* (flowers), g. *P. timoriana* (tree), h. *D. dulcis* (tree), i. *D. kutejensis* (fruits), j. *B. angulata* (fruits) and k. *B. costulata* (tree).

Material and Methods

Chemicals

p-nitrophenyl-alpha-D-glucopyranoside (pNPG) was purchased from Sigma-Aldrich (St Louis, MO, USA). Sucrase was purchased from Junsei Chemical Co., Ltd (Tokyo, Japan). α-glucosidase from yeast (*Saccharomyces cerevisiae*) EC. 3. 2. 1. 20 was purchased from Wako Chemicals (Osaka, Japan). Rat intestinal acetone powder was purchased from Sigma-Aldrich (St Louis, MO, USA). Glucose C-II Test kit was purchased from Wako Chemicals (Osaka, Japan).

Plants Material and Extraction

Sample Collection

Plants sample was collected in Kuala Buayan village, Meliau Distric, Sanggau Regency, West Kalimantan Province, Indonesia. The plant part collected is wood bark (the bark position in the tree is upper 1 m high from the ground). The wood bark cleaned from the outer skin to avoid contamination such as a dirt or moss, and small square-shaped pieces like a chip were made and then dried in the air for 4 weeks. Fifty grams of dried wood bark were grounded to get fine powder using an electric grinder (Oster, Sunbeam Products, Inc). They were kept in plastic bags until further use. The voucher specimens of the plants were made for identifying the scientific name and deposited at the Laboratory of Wood Technology, Tanjungpura University Pontianak.

Extraction

Thirty grams of bark powder were extracted three times with 100 ml of methanol (99.7%) using a soxhlet extractor (Yamato Water Bath BS660, Yamato Scientific Co.Ltd) for 1 hour at temperature 70℃. This extract was filtered through a Whatman filter paper (No. 2) and the filtrate was collected, evaporated in a vacuum rotary evaporator (Eyela N-1000, Tokyo, Japan) at 40℃ with a rotary speed at 5 rpm, dried for one day in a wind dryer (Pierce, Reacti-Therm and Reacti-Vap, Thermo Fisher Scientific Inc., Waltham, MA), and dried for one day in a vacuum dryer (Ettas, AVO-250NB, Active Co., Saitama, Japan) to obtain final residues. The percentage of the methanol extract content was calculated.

α-glucosidase Assays

The α-glucosidase inhibition assay using purified enzyme derived from yeast (*Saccharomyces cerevisiae*) and rat intestinal acetone powder containing sucrase substrate (rat intestinal sucrase). Yeast and rat intesitinal is different source of enzyme (Tadera *et al*., 2006). Yeast from *S. cerevisiae* with code EC. 3. 2. 1. 20 contains maltase (Yamamoto *et al*., 2004), and sucrase substrate used in most studies on the rat intestinal α-glucosidase inhibitory activity (Tadera *et al*., 2006). Differences in enzyme source will affect the inhibitory effects on α-glucosidase activity.

a. Yeast (*S. cerevisiae*) with pNPG substrate

The yeast α-glucosidase activity measured according to Bothon *et al*., (2013) with slight modification. The assay was performed by adding 10 μl of phosphate buffer 0.1 mol/l (pH6. 8), 150 μ1 of p-nitrophenyl-alpha-D-glucopyranoside (pNPG) (5 mmol/ml), 20 μl of extracts with varying concentrations (200, 20, 15, 10, 5, 2, 1 μg/ml) and 20 μl of α-glucosidase enzyme (5μg/ml) in a 96 well plate. The change in absorbance (Multiskan JX, Thermo Electron Co., Waltham, MA) at 405 nm was recorded at one minute interval for 10 minutes. All determinations were made in triplicate. The α-glucosidase inhibitory activity was calculated with the following equation:

$$Inhibition\ (\%) = \left(1 - \frac{Slope\ of\ Absorbance\ Treatment}{Slope\ of\ Absorbance\ Control}\right) x\ 100\%$$

IC_{50} value was calculated using a linear regression analysis. IC_{50} is concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay condition.

b. Rats intestinal acetone powder with sucrase substrate (rat intestinal sucrase)

The rat intestinal sucrase activity was determined according to Ikarashi *et al*.,(2012)with slight modifications. Five hundred mg of rat intestinal acetone powder dissolved in 5 ml of phosphate buffer 0.1 mol/l (pH 6.8) and then centrifuged at 2500 rpm for 5 minutes. The supernatant was used as a sucrase (one of the α-glucosidase) solution. The α-glucosidase inhibition assay was performed using a 96 well plate. Each well of the 96 well plate contains 10 μl of phosphate buffer 0.1 mol/l (pH 6.8), 150 μl sucrase (5 mg/ml), 20 μl of extract with two concentration (500 and 1000 μg/ml) and 20 μl of α-glucosidase enzyme (100 mg/ml). Reaction mixture was incubated in an incubator (Low Temperature O_2/CO_2 Incubator, Wakenyaku, Japan) at temperature 37℃ for 30 minutes, heat up on the Cool-Hotter Dry Bath Incubator (MS, Major Science) at temperature 70℃ for 3 minutes. Twenty μl of each reaction mixture was transferred to another well of a 96 well plate. After adding 150 μl of glucose C-II test substrate solution, the plate was incubated at room temperature for 15 minute. The change in absorbance (Multiskan JX, Thermo Electron Co., Waltham, MA) at 492 nm was recorded at 1 minute. All determinations were performed in triplicate. The α-glucosidase inhibitory activity was calculated with the following equation:

$$Inhibition\ (\%) = \left(1 - \frac{Absorbance\ Treatment}{Absorbance\ Control - Absorbance\ blank}\right) x\ 100\%$$

IC_{50} value was calculated using a linear regression analysis.

Result and Discussion

The efficiency of the methanol extraction from wood bark

In this study, we measure the amount of materials extracted by methanol from wood bark of *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* plants family. Results of the research showed that extractive contents vary among species as shown in Figure 2.

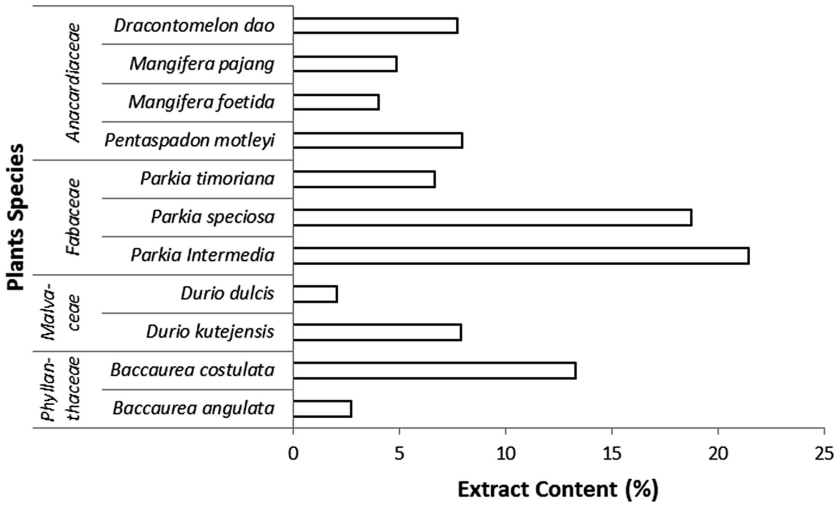

Figure 2. The content (%) of wood bark methanol extract of species of *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* plants family.

The extractive contents varied from 2.05 to 21.48% among these species. Based on the classification of Indonesia classes of wood chemical component (extracted contents: < 2% lower, 2-4% moderate, and > 4% higher) (Departemen Pertanian, 1976). Almost all of the species have extractive contents more than 4%. Especially, *P. intermedia* (Fabaceae) had 21.48%. In contrast, *D. dulcis* (Malvaceae) and *B. angulata* (Phyllathaceae) had 2.05 and 2.75% content, respectively.

The proportion of extractive contents generally varies depending on the species. Extractive contents range from less than 1% to more than 10% and those of tropical wood species were around 20% (Tsoumis, 1991). Different parts of the same tree such as branches, stems, roots, bark and leaves usually have differences at the levels and in the composition of the amounts of extractive contents (Sjostrom, 1981).

Methanol was used as the extraction solvent because of its high efficiency to extract materials. Methanol efficiently penetrates in the cell membrane, in order to obtain endocellular components (Silva *et al*., 1998). Extraction process using polar solvent such as methanol, ethanol and water is very effective to isolate bioactive compounds (Filho, 2006).

Many kinds of bioactive compounds isolated from secondary metabolites of medicinal plants have high inhibition on α glucosidase activity such as alkaloids, phenolic, flavonoids, isoflavone, flavonolignans, flavanone, flavonols, anthocyanins, anthraquinones, anthrones, xanthones, glycosides, feruloylglucosides, acetophenone glucosides, stilbenoids, terpenoids, triterpenoids, curcuminoids, lignan, acids, phytosterols and myoinositol (Benalla *et al*., 2010; Kumar *et al*. 2011).

The bioactive compounds from all species especially from wood bark that already extracted are not yet identified. In case of methanol extracts, however, some of compounds are identified such as oils, fats, waxes, alkaloids, flavones, polyphenols, tannins, saponins, glycosides and aglycones (Houghton and Raman, 1998, Filho, 2006). Based on the Table 1, another parts (fruits, leaf, wood) of plants species that were already extracted are known to have bioactive compounds as medicine, and extracted materials from wood bark are expected to have bioactive compounds and allegedly have anti-diabetic activity, especially by inhibiting α-glucosidase.

α-glucosidase inhibitory effect of methanol extracts on yeast and rat intestinal sucrase.

In this study, we examined the effects of wood bark methanol extracts of plant species from *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* plants family on the α-glucosidase activity, which is the target of diabetic type 2 therapy drugs. High blood sugar level in diabetic patients due to rapid absorption of glucose such

as maltase and sucrase in small intestinal by α-glucosidase, and inhibition of these enzyme will decrease high blood sugar level (Jo *et al*., 2010). In this report, we used two kinds of α-glucosidase enzyme, yeast with contain maltase and rats intestinal contain sucrase substrate.

As shown in Fig. 3A, all of plants species of methanol extracts from *Anacardiaceae* family have strong activity to inhibit yeast α-glucosidase. Methanol extract from *D. dao* inhibited 66.11% of the enzyme at the concentration of 5 μg/ml. This activity is higher than that from *P. motleyi* (10μg/ml: 62.01%), *M. pajang* (15μg/ml: 70.32%) or *M. foetida* (15 μg/ml: 59.24%). As shown in Fig. 3B, methanol extract only from *P. motleyi* inhibited 52.82% of the rat intestinal sucrase activity at the concentration of 1000 μg/ml, whereas other species inhibited less than 50% of the enzyme activity.

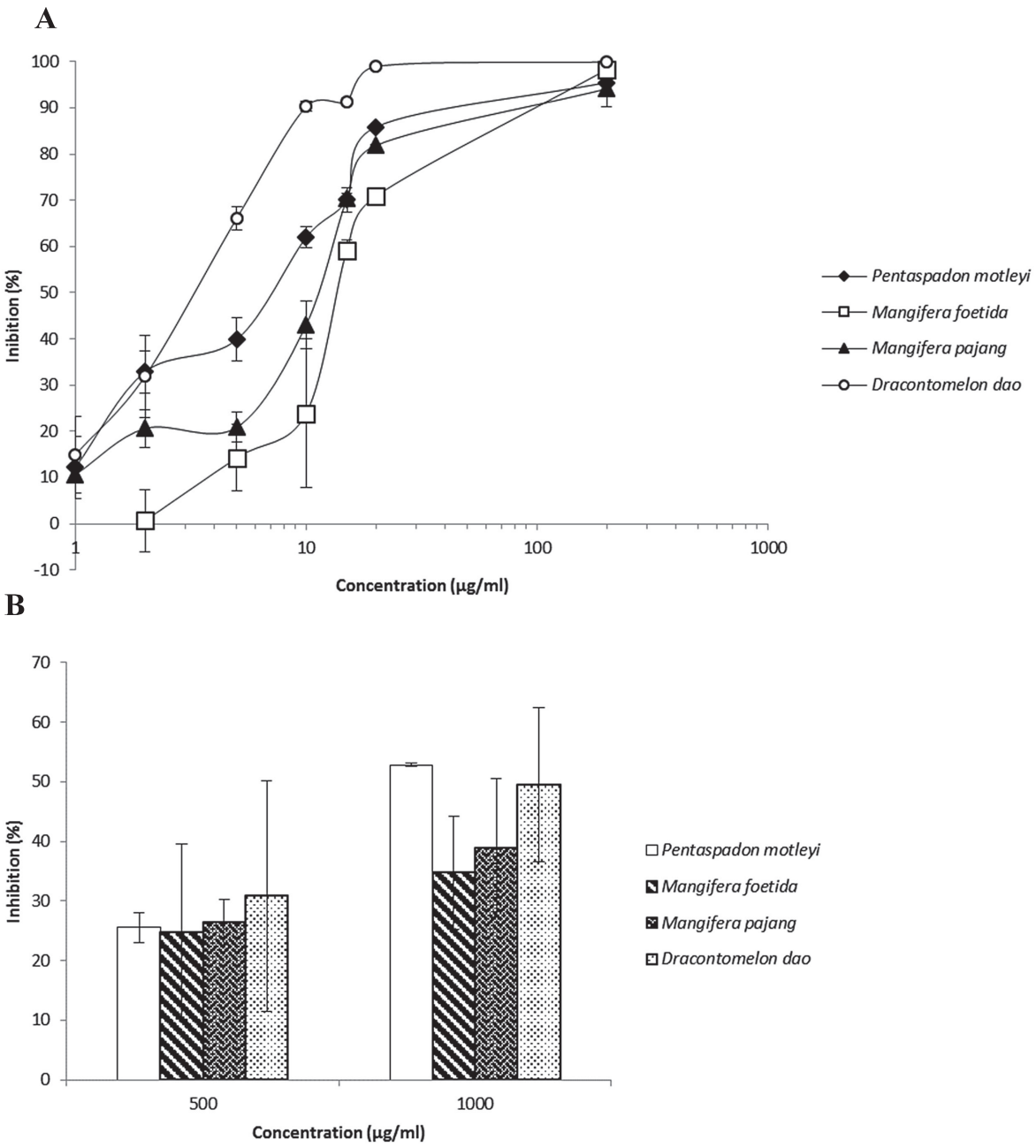

Figure 3. Inhibition of α-glucosidase by methanol extracts from the wood bark of *Anacardiaceae* plants family.
(A) Inhibition on yeast enzyme and (B) Inhibition on rat intestinal sucrase. The values are shown in mean ± SD. (n = 3)

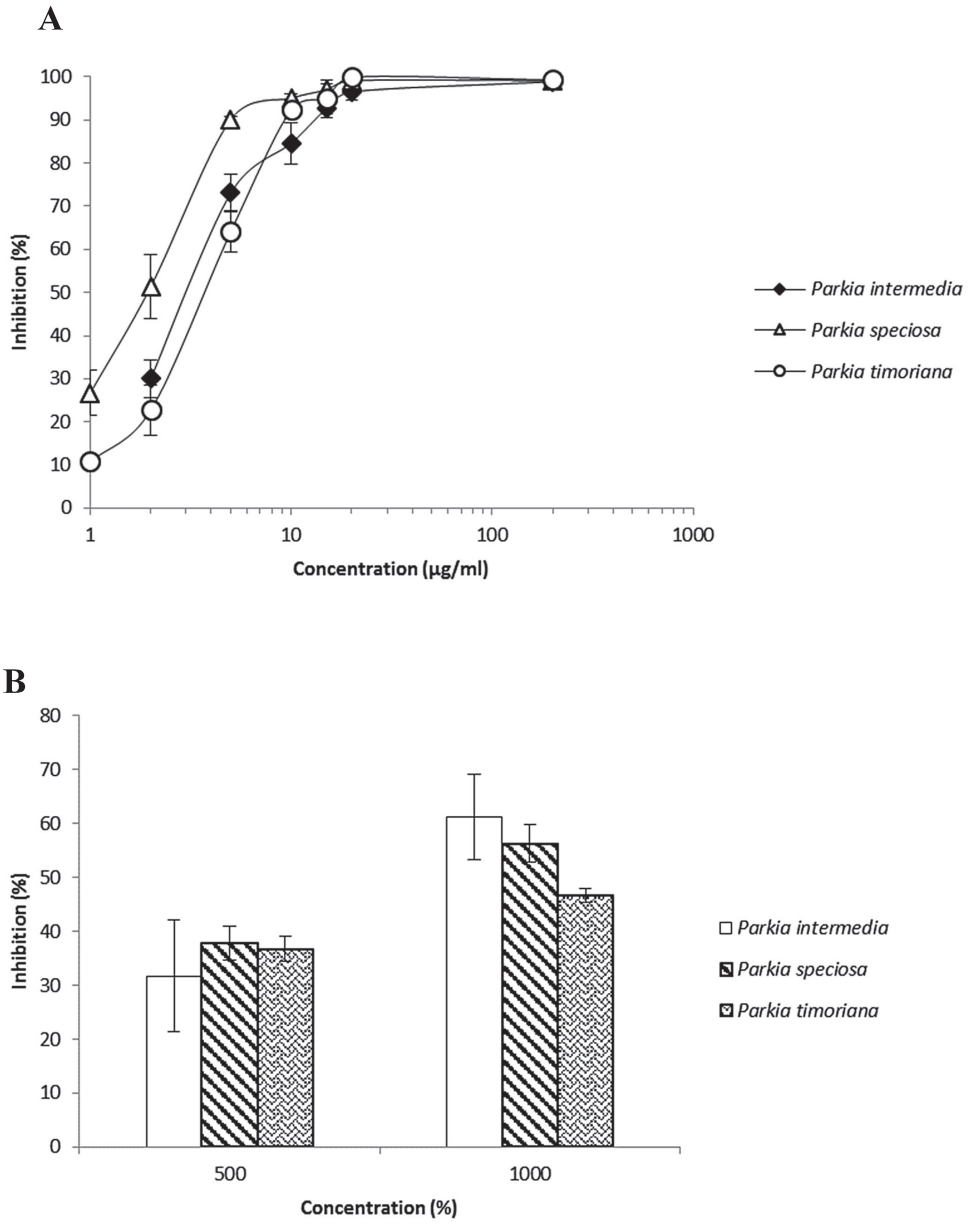

Figure 4. Inbibition of α-glucosidase by methanol extracts from the wood bark of *Fabaceae* plants family. (A) Inhibition on yeast enzyme and (B) Inhibition on rat intestinal sucrase. The values are shown in mean ± SD. (n = 3)

In Fig. 4A, methanol extracts from the wood bark of *Fabaceae* plants family, especially *Parkia* species have a strong activity to inhibit yeast α-glucosidase. *P. speciosa* extracts inhibited 51.36% of the enzyme activity at the concentration of 2μg/ml. This is higher than that of *P. intermedia* (5μg/ml: 73.08%) and *P. timoriana* (5μg/ml: 64.11%). However, in Fig. 4B, at concentration 1000μg/ml, methanol extract from *P. intermedia* inhibited 61.29% and *P. speciosa* inhibited 56.32% of the rat intestinal sucrase activity. The methanol extract from *P. timoriana* had lower inhibitory effect on rat intestinal sucrase.

As shown in Fig. 5A, methanol extract from the wood bark of *D. kutejensis* inhibited 63.82% of the yeast α-glucosidase activity at the concentration of 5μg/ml and that from *D. dulcis* inhibited 61.90% of the enzyme activity at the concentration of 15 μg/ml. In contrast, as shown in Fig. 5B, methanol extracts only from *D. kutejensis* and *D. dulcis* inhibited less than 50% of rat intestinal sucrase at the concentration of 1000 μg/ml.

As shown in Fig. 6A, methanol extract from the

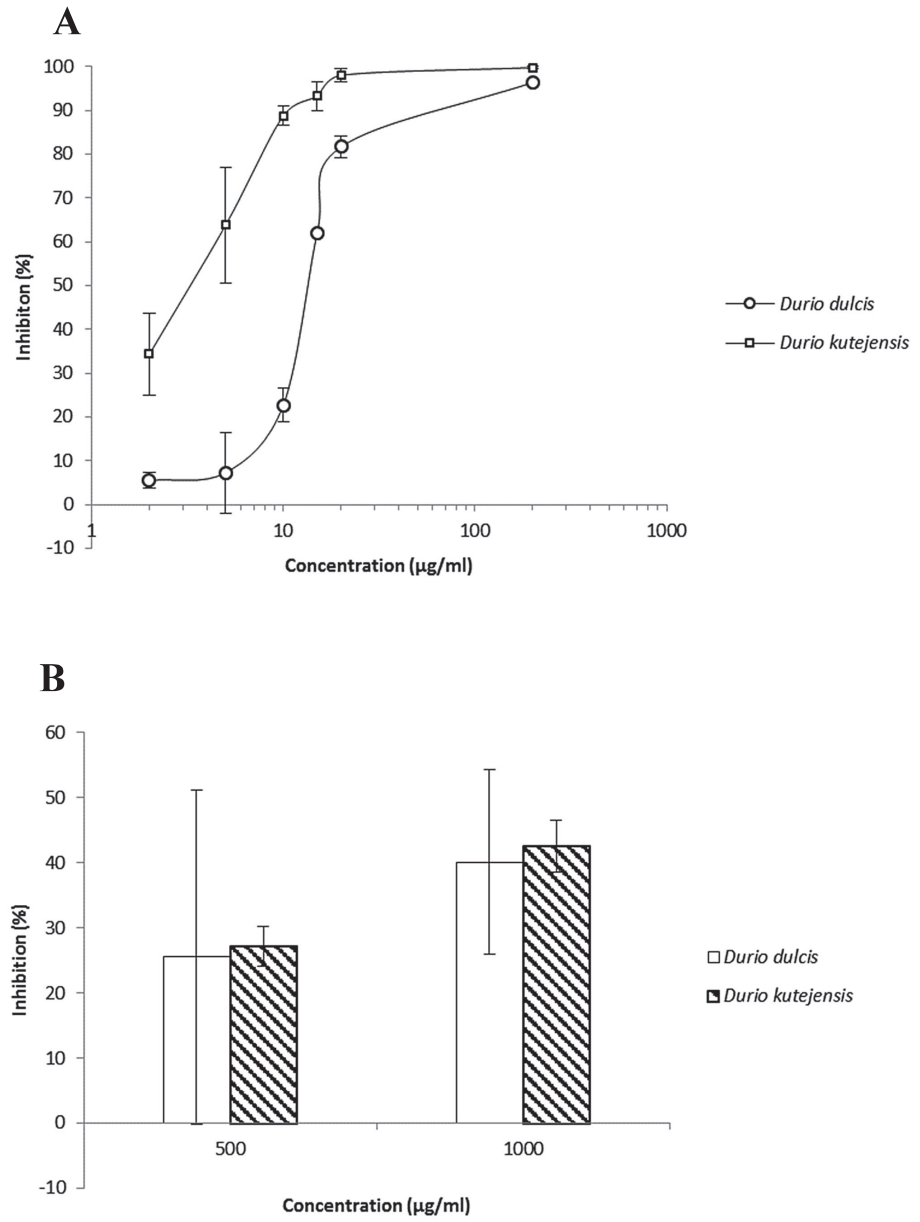

Figure 5. Inhibition of α-glucosidase by methanol extracts from the wood bark of *Malvaceae* plants family. (A) Inhibition on yeast enzyme and (B) Inhibition on rat intestinal sucrase. The values are shown in mean ± SD. (n = 3)

wood bark of *B. costulata* inhibited the 76.81% of the yeast α-glucosidase activity at the concentration of 15 µg/ml. In contrast, that from *B. angulata* inhibited 31.81% of the enzyme activity at the concentration of 200µg/ml. As shown in Fig. 6B, methanol extract from the wood bark of *B. costulata* inhibited 51.36% of rat intestinal sucrase at the concentration of 1000 µg/ml, whereas *B. angulata* inhibited 24.21% of the enzyme activity.

Differences in distribution of extractive substances in each extract from individual plant species affect the level of inhibition of α-glucosidase. It is suggested that higher the level concentration of bioactive compound results in higher level of inhibition α-glucosidase activity. According to Kardono (2003), various level of α-glucosidase inhibition in medicinal plant is due to differences in bioactive compound contained in a plant.

Yeast α-glucosidase enzymes were more susceptible

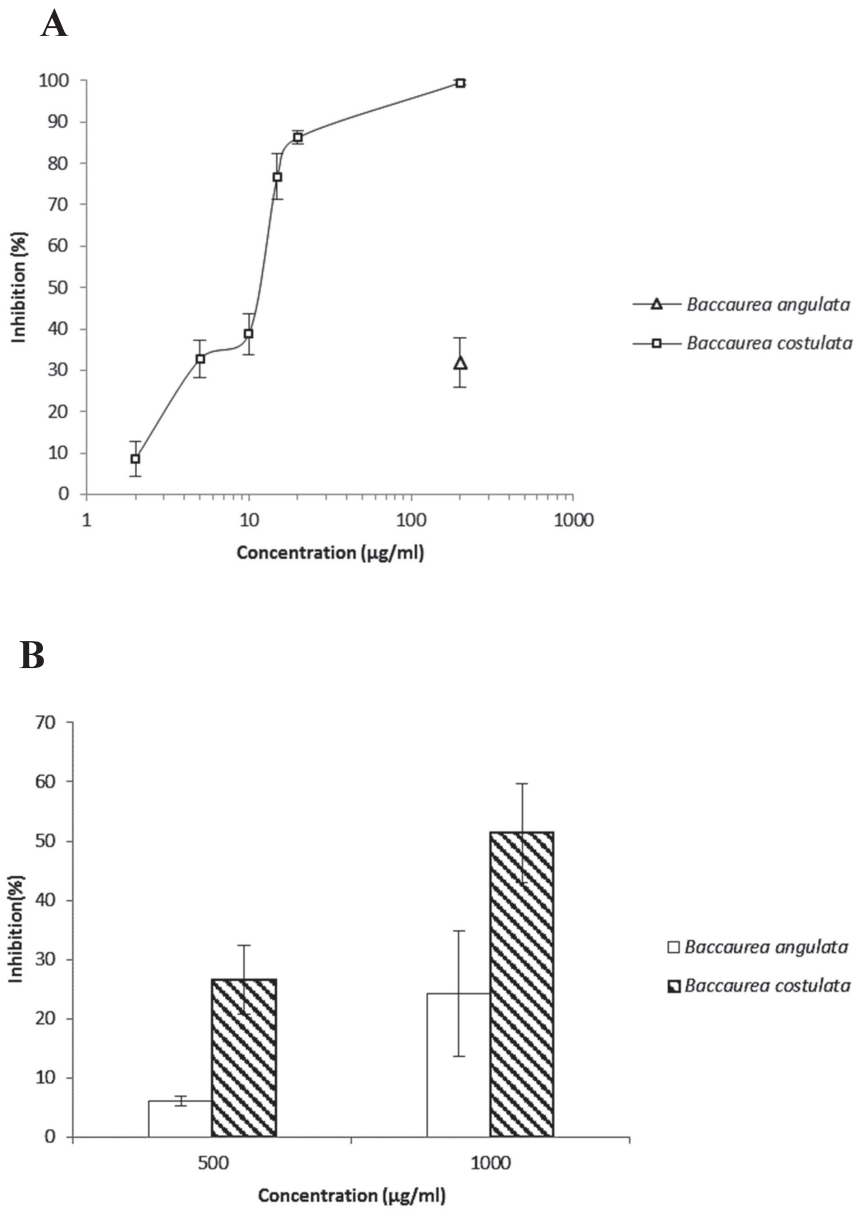

Figure 6. Inhibition of α-glucosidase activity by methanol extracts from the wood bark of *Phyllanthaceae* plants family. (A) Inhibition on yeast enzyme and (B) Inhibition on rat intestinal sucrase. The values are shown in mean ± SD. (n = 3)

than rat intestinal sucrase to wood bark methanol extracts of plant species from *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* plants family. Acarbosa as commercial anti-diabetic drug had high inhibitory activity on mammalian α-glucosidase and low inhibitory activity on yeast α-glucosidase, and quercetin has good ability to inhibit yeast α-glucosidase (Eyla *et al*., 2012). Result of our research showed that methanol extracts from almost all the species have high inhibitory effects on yeast α-glucosidase activity except from that from *B. angulata* (Phyllanthaceae). In contrast, methanol extracts from most plant species had much lower inhibitory effect on the activity of rat intestinal sucrase. *Fabaceae, Phyllanthaceae* and *Anacardiaceae* showed inhibitory activity above 50% on rat intestinal sucrase at the concentration of 1000 µg/ml.

Currently, it is already known that some of the medicinal plants such as *Anacardiaceae, Fabaceae* and *Malvaceae* family have anti-diabetic activity. Simmond and Howes (2006) reported that 10 species of

Anacardiaceae, 78 species of *Fabaceae* and 11 species of *Malvaceae* have a function of anti-diabetes, with plants part used is leaf, bark, seed, stem, all part, nut, root, flower, fruits, wood and bulb. From these all species, only fruit part of *P. speciosa* species that is already reported to have a function as anti-diabetes. In this study, we reported that methanol extracts from wood bark of *Anacardiaceae, Fabaceae*, *Malvaceae* and *Phyllanthaceae* plants family have anti-diabetic activity. According to Patel *et al*., (2012), several plants in different family have been used as anti-diabetic medicinal medicine, and one of the most potential plant to decrease blood glucose is *Fabaceae.* It is relevant with the results of our research, where all *Parkia* species (Fabaceae) tested have high inhibitory activity against yeast α-glucosidase.

In order to find bioactive compounds from plants, functional materials can be traced by three approaches, that is random, ethnopharmacology and taxonomy (Filho, 2006). In this study, to identify the plants as anti-diabetic we used two approaches, ethnopharmacology and taxonomy. In ethno- pharmacology, among 11 plant species studied, traditionally used as an anti-diabetic medicine are only 4 species (*Fabaceae* and *Phyllanthaceae*). In the taxonomic approach, plants species from *Malvaceae* and *Anacardiaceae* are useful as anti-diabetic, and we try to examine 7 other species as anti-diabetic. Results of this study showed that almost all species have activity in inhibiting of yeast α-glucosidase, except *B. angulata,* which has lower activity in both tests using yeast or rat intestinal sucrase.

Based on the α-glucosidase inhibition assays, we calculated IC_{50} of each species extracts. IC_{50} is the concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay condition. In this study, some plants inhibited less than 50% of α-glucosidase activity at the maximum concentration. In this case, the IC_{50} values of these plant extracts were not calculated.

As shown in Fig. 7A, three species of *Parkia, P. speciosa, P. intermedia* and *P. timoriana* have strong inhibitory effects on yeast α-glucosidase activity, because they are able to inhibit 50% of α-glucosidase activity at lower concentrations compared with other species. For species in other family, *Anacardiaceae, Malvaceae,* and *Phyllanthaceae*, IC_{50} values are diversified. In contrast, species of *D. dao* and *D. kutejenis* have IC_{50} values similar to *Parkia* species showing strong inhibitory effects on yeast α-glucosidase activity. It is reported that ethanol extracts from *Symplocos cochinchinensis* has IC_{50} value of 82.07 ± 2.10 μg/ml (Antu *et al*., 2014). All species in our research have lower IC_{50} values than that of *Symplocos cochinchinensis* in terms of inhibition on yeast α-glucosidase activity. In contrast, in the assay with rat intestinal sucrase (Fig. 7B), *P. timoriana* has the lowest IC_{50} values among those from four species.

Differences enzyme source in this case is yeast derived from *S. cerrevisae* (EC.3.2.1.20) with the main contain is maltase and rat intestinal acetone powder with its substrate is sucrase (EC.3.2.1.48), showed differences in the value of inhibitory activity, with IC_{50} value of yeast α-glucosidase more lower than rat intestinal sucrase. These results are relevant with studies conducted by Tadera *et al*., (2006) that reported α-glucosidase inhibitory activity of six groups of flavonoids compounds especially flavonol, flavanone, isoflavone and anthocyanidin against yeast α-glucosidase (200 μM : inhibition 61-99%) is higher than the rat small intestinal α-glucosidase (0. 5mM : inhibition < 32%). In addition, Jo *et al.*, (2010) reported quercetin compound have higher inhibitory activity against rat intestinal maltase rather than rat intestinal sucrase.

The use of polar solvents such as methanol in extracting the wood bark will get phenolic constituents in very large amounts, such as flavanoid compounds included in the group of condensed tannins (phenolic acids) and monomer of flavonoids such as quercetin and dihydroquercetin (taxifolin) (Sjostrom, 1981). Methanol extract of wood bark that used in this research allegedly contains flavonoids and quercetin as bioactive compounds. It is shown by the strong inhibitory activity against yeast α-glucosidase contain maltase rather than rat intestinal sucrase. Plants species of *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* in this research, allegedly have good ability to delaying maltase absorption rather than sucrase in small intestinal of diabetic patients. The major absorbable glucose as digestive product of carbohydrates in the small intestine is maltase (Tadera *et al*., 2006), and delaying of maltase

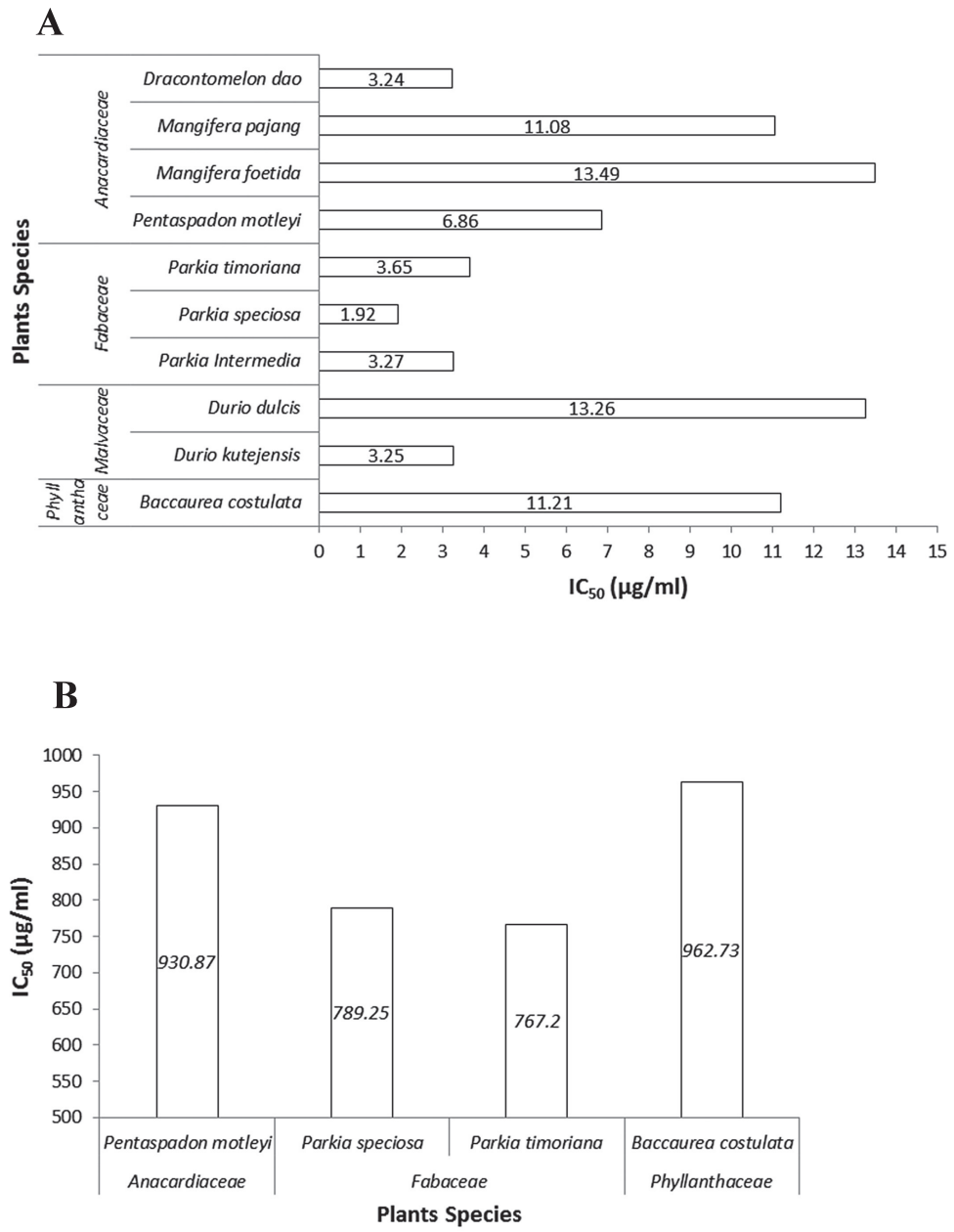

Figure 7. IC_{50} of α-glucosidase inhibition. (A) IC_{50} Inhibition on yeast enzyme and (B) IC_{50} Inhibition on rat intestinal sucrase

absorption in small intestinal will decrease the rate of glucose absorption resulting in the reduction of blood glucose level of diabetic patients.

Local people in West Kalimantan Indonesia traditionally using roots as a plants part of medicinal plants for diabetes therapy medicine. Around a handful of roots, extracted using water (boiled) and drink it one or three times a day. In this research, we used methanol for extracting wood bark and result show that methanol extracts have ability to inhibit yeast α-glucosidase with low concentration and almost all of these plants is very potential for anti diabetes medicine.

In this study, the mechanism of inhibition is not yet known. So, it is necessary to research further to determine the mechanism of α-glucosidase inhibition by potential plant extracts. In addition, it is necessary to examine the effects of these plant extracts *in vivo* so that we can estimate the effectiveness of these medicinal plants to

inhibit α-glucosidase before applying to humans, especially to patients with type 2 diabetes mellitus.

Conclusion

We examined the inhibitory effect of wood bark methanol extract from plant species of *Anacardiaceae, Fabaceae, Malvaceae* and *Phyllanthaceae* plants family on α-glucosidase activity using two kinds of α-glucosidase enzyme, yeast α-glucosidase and rat intestinal sucrase. As a result, we found that almost all the species have high inhibitory activity on yeast α-glucosidase, except *B. angulata* (Phyllanthaceae) that has much lower level of inhibition. Inhibitory effects of these plant extracts on rat intestinal sucrase activity are much lower compared with those on yeast α-glucosidase. Only a few species, especially from the family of *Fabaceae* (*P. intermedia* and *P. speciosa*), *Phyllanthaceae* (*B. costulata*) and *Anacardiaceae* (*P. motleyi*) inhibited more than 50% of rat intestinal sucrase activity at the concentration of 1000 μg/ml. Further study is required to clarify the mechanism of the inhibition of α-glucosidase by using animal model of type 2 diabetes mellitus. Purification and identification of bioactive compound from these plants is also necessary to apply to human type 2 diabetes patients.

Conflict of Interest

The authors declare that there is no conflict of interest

Acknowledgment

Thank you for DIKTI that provide Scholarship, PIU IDB 7 in 1 of Tanjungpura University, Mr. Akira Tominaga, Mr. Sota Tanaka, Mr. Yoshiaki Iiguni for support and suggestion, and people of Kuala Buayan village especially Supriono that help us to collect the sample.

References

Ablat A, Mohamad J, Awang K, Shilpi JA and Arya A. 2014. Evaluation of antidiabetic and antioksidan properties of *Brucea javanica* seed. *The Scientific World Journal* 2014: 1-8.

Ahmed IA, Mikail MA, Ibrahim M, Hazali N, Rasad MSBA, Ghani RA, Wahab RA, Arief SJ and Yahya MNA. 2014. Invitro antioxidants properties of underutilized *Baccaurea angulata* Fruits. *International Journal of Advances in Agricultural and Environmental Enggenering* (IJAAEE) 1: 144-150.

Ajibola M, Olugbemi O, Stephanie A, Joseph D and Denen A. 2013. Hepatoprotective effect of *Parkia biglobosa* stem bark methanolic extract on paracetamol induced liver damagein Wistar Rats. *American Journal of Biomedical and Life Sciences* 1: 75-78.

Al Batran R, Al-Bayaty F, Jamil Al-Obaidi MM, Abdualkader AM, Hadi HA, Ali HM, and Abdulla MA. 2013. In vivo antioxidant and antiulcer activity of *Parkia speciosa* ethanolic leaf extract against ethanol-induced gastric ulcer in rats. *PLoS ONE* 8: 1-11.

Antu KA, Riya MP, Mishra A, Anilkumar KS, Chandrakanth CK, Tamrakar AK, Srivastaka AK and Raghu KG. 2014. Antidiabetic property of *Symplocos cochinchinensis* is mediated by inhibition of alpha glucosidase and enhanced insulin sensitivity. *PLoS ONE* 9: 1-13.

Arung ET, Suwinarti W, Hendra M, Supomo, Kusuma IW, Puteri DCN, Eroglu HA, Kim Y, Shimizu K and Ishikawa H. 2015. Determination of antioxidant and anti-melanogenesis activities of Indonesian Lai, *Durio kutejensis* [Bombacaceae (Hassk) Becc] fruit extract. *Tropical Journal of Pharmaceutical Research* 14: 41-46.

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

Bothon FTD, Debiton E, Avlessi F, Forestier C, Teulade JC, and Sohounhloue DKC. 2013. In vitro biological effects of two anti-diabetic medicinal plants used in Benin as folk medicine. *BMC Complementary & Alternative Medicine.* 13 : 1-8.

Chaverri JP, Rodríguez NC, Ibarra MO and Rojas JMP. 2008. Medicinal properties of mangosteen (*Garcinia mangostana*). *Food and Chemical Toxicology* 46: 3227−3239.

Departemen Pertanian. 1976. *Vademikum Kehutanan Indonesia.* Direktorat Jendral Kehutanan, Jakarta.

Dicker D. 2011. DPP-4 Inhibitor, impact on glycemic control and cardiovascular risk factor. *Diabetes Care* 34: S276-S278.

Eyla B, Malik A, Septimahani PI and Loranza B. 2012. Antidiabetic activity test by inhibition of α-glucosedase and phytochemical screening from the most active fraction of buni (*Antidesma bunius* L) stem bark and Leaves. *International Journal of PharmTech Research* 4: 1667-1671.

Filho RRM. 2006. Bioactive phytocompounds : new approaches in the phytosciences. Pp 1-24 in Iqbal A *et al.*, (eds) *Modern Phytomedicine*. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Hasan MR, Uddin N, Hossain MM, Hasan M, Yousuf ME, Lopa SS, Rahman T and Choudhuri MSK. 2014. Invitro α-amylase inhibitory activity and invivo hypoglycemic effect of ethyl acetate of *Mollatus repandus* (Willd) Muell. in stem in rat model. *Journal of Coastal Life medicine* 2: 721-726.

Hassan FA, Ismail A, Abdulhamid A and Azlan A. 2011. Identification and quantification of phenolic compounds in bambangan (*Mangifera pajang* Kort) peels and their free radical scavenging activity. *Journal of Agricultural and Food Chemistry* 59: 9102-9111.

Haughton PJ and Raman A. 1998. *Laboratory Handbook for the Fractionation of Natural Extracts.* Chapman & Hall, London.

Ikarashi N, Takeda R, Ito K, Ochiai W and Sugiyama K. 2011. The inhibition of lipase and glucosidase activities by Acacia polyphenol. *Evidence-Based Complementary and Alternative Medicine* 2011, 1-8.

Infodatin. 2014. *Situasi dan Analisis Diabetes.* Pusat Data dan Informasi Kementerian Kesehatan RI, Jakarta.

International Diabetes Federation (IDF). 2014. *IDF Diabetes Atlas, 6th Edition Revision 2014*. International Diabetes Federation, Brussels.

Jaiswal N, Srivastava SP, Bhatia V, Mishra A, Sonkar AK, Narender T, Srivastava AK, and Tamrakar AK. 2012. Inhibition of alpha-glocosidase by *Acacia nilotica* prevents hyperglycemia along with improvement of diabetic complications via aldose reductase inhibition. *Journal Diabetes & Metabolism* S6: 1-7.

Jamaluddin F and Mohamed S. 1993. Hypoglicemic effect of extracts of petai papan (*Parkia spesiosa,* Hassk). *Pertanika J.Trop.Agric.Sci* 16: 161-165.

Jamaluddin F, Mohamed S, and Lajis MN. 1995. Hypoglycaemic effect of stigmast-en-3-one, from *Parkia speciosa* empety pods. *Food Chemistry* 54: 9-13.

Jauhari NK, Ibrahim D, Ibrahim M, Yahya MNA, Nor NM, Isa KAM, Ayob MK, Omar MN and Hazali N. 2013. Proximate composition and antioxidants activity of dried belimbing dayak (*Baccaurea angulata*) Fruits. *Sains Malaysiana* 42: 129-134.

Jo SH, Ka EH , Lee HS , Apostolidis E , Jang HD and Kwon YI. 2010. Comparison of antioxidant potential and rat intestinal α-glucosidases inhibitory qctivities of quercetin, rutin, and isoquercetin. *Journal of Applied Research in Natural Products* 2: 52-60.

Kardono LBS. 2003. Kajian Kandungan Kimia Mahkota Dewa (*Phaleria macrocarpa*). Pp 72-76 in Pusat Peneltian dan Pengembagan Farmasi dan Obat Tradisional Departemen Kesehatan (eds) *Proceeding of Pameran Produk Obat Tradisional dan Seminar Sehari Mahkota Dewa.* Agustus 2003. Jakarta.

Kashtoh H, Hussain S, Khan A, Saad SM, Khan JAJ, Khan KM, Perveen S and Choudhary MI. 2014. Oxadiazoles and thiadiazoles: novel a-glucosidase inhibitors. *Bioorganic & Medicinal Chemistry* 22: 5454−5465.

Khan MR and Omoloso AD. 2002. Antibacterial and antifungal activities of *Dracontomelon dao. Fitoterapia* 73: 437-330.

Khoo HE, Prasad KN, Ismail A and Esa NM. 2010. Carotenoids from *Mangifera pajang* and their antioxidant capacity. *Molecules* 15: 6699-6712.

Klangjareonchai T and Roongpisuthipong C. 2012. The effect of *Tinospora crispa* on serum glucose and insulin levels in patients with type 2 diabetes mellitus. *Journal of Biomedicine and Biotechnology* 2012: 1-4.

Kumar S, Narwal S, Kumar V and Prakash O. 2011. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn Rev,* 5 : 19−29.

Mirfat AHS, Razali M and Salma I. 2013. Antioxidant properties of wild *Mangifera* species in Malaysia. *Acta Horticulturae* 979: 651-660.

Okoli CO, Ibiam AF, Ezike AC, Akah PA and Okoye TC. 2010. Evaluation of antidiabetic potentials of *Phyllanthus niruri* in alloxan diabetic rats. *African Journal of Biotechnology* 9: 248-259.

Oyedemi SO, Adewusi EA, Aiyegoro OA and Akinpelu DA. 2011. Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic wistar rats. *Asian Pacific Journal of Tropical Biomedicine* 1: 353-358.

Patel DK, Kumar R, Laloo D and Hemalatha S. 2012. Natural medicines from plant source used for therapy of diabetes mellitus: an overview of its pharmacological aspects. *Asian Pacific Journal of Tropical Disease* 2: 239-250.

Rudiyansyah and Garson MJ. 2006. Secondary metabolites from the wood bark of *Durio zibethinus* and *Durio kutejensis. Journal of Natural Product* 69: 1218-1221.

Silva GL, Lee IK and Kinghorn AD. 1998. Special problems with the extraction of plants. Pp 343-364 in Cannell RJP (eds) *Natural Products Isolation.* Humana Press lnc, New Jersey.

Simmonds MSJ and Howes MJR. 2006. Plants used in the treatment of diabetes. Pp 19-82 in Amala Soumyanath (eds) *Traditional Herbal Medicines for Modern Times Antidiabetic Plants.* CRC Press Taylor & Francis Group, Boca Raton.

Sjostrom E. 1981. *Wood Chemistry.* Academic Press. New York.

Sulaiman SH and Ooi KL. 2014. Antioxidant and α-glucosidase inhibitory activities of 40 tropical juices from Malaysia and identifìcation of phenolics from the bioactive fruit juices of *Barringtonia racemosa* and *Phyllanthus acidus. Journal of Agricultural and Food Chemistry* 62: 9576-9585.

Soenanto H. 2005. *Musnahkan Penyakit dengan Tanaman Obat.* Penebar Swadaya. Jakarta.

Tadera K, Minami Y, Takamatsu K and Matsuoka T. 2006. Inhibition of α-glucosidase and α-amylase by Flavanoids. *J Nutr Sci Vitaminol* 52 : 149-153.

Tisnadjaja D, Hidayat SL, Sumirja S and Simanjuntak P. 2006. Pengkajian kandungan fitosterol pada tanaman kedawung (*Parkia roxburgii* G. Don.). *Biodiversitas* 7: 21-24.

Tsoumis G. 1991. *Science and Technology of Wood.* Van Nostrand Reinhold, New York.

Tyug TS, Johar MH and Ismail A. 2010. Antioxidant properties of fresh, powder, and fiber products of mango (*Mangifera foetida*) fruit. *International Journal of Food Properties* 13: 682-691.

Xu W, Zhou Q, Yin J, Yao Y and Zhang J. 2014. Anti-diabetic effects of polysaccharides from *Talinum triangulare* in streptozotocin (STZ)-induced type 2 diabetic male mice. *International Journal of Biological Macromolecules* 72: 575-579.

Yamamoto K, Nakayama A, Yamamoto Y and Tabata S. 2004. Val216 decides the substrate specificity of α-glukosidase in *Saccharomyces cerevisae. Eur. J. Biochem* 271 : 3414-3420.

Yusro F, Syafii W and Pribadi ES. 2010. Sifat anti cendawan *Trichophyton mentagrophytes* dan *Candida albicans* dari zat ekstraktif kayu pelanjau (*Pentaspadon motleyi* Hook.f). *Jurnal Ilmu Kehutanan* IV: 57-69.

Yusro F, Diba F, Mariani Y, Mulyadi, Johansyah and Ohtani K. 2015. Traditional knowledges of Dayak ethnic in West Kalimantan Indonesia to treat diabetic and cancer diseases. Pp 141-147 in Lukmandaru G *et al.*, (eds) *Proceeding of the National Seminar : Peranan dan Strategi Kebijakan Pemanfaatan Hasil Hutan Bukan Kayu (HHBK) Dalam Meningkatkan Daya Guna Kawasan (Hutan).* 6-7 November 2014, Yogyakarta.

Zang S and Li XZ. 2015. Inhibition of α-glucosidase by polysaccharides from the fruit hull of *Camellia oleifera* Abel. *Carbohydrate Polymer* 115: 38-43.