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Research Paper

Alpha Glukosidase Inhibitory Test and Total Phenolic Content of Ethanol Extract of *Parkia Speciosa* Plant

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Abstract
Parkia speciosa (Fabaceae), much grow in South Sumatera. The seed of *P. speciosa* used as traditional medicine for diabetes mellitus therapy. Another parts of *P speciosa* is suspected to have the same chemical compounds and potency as the seed. Based on phytochemical screening of leaf and rind of *P speciosa* have secondary metabolites as flavonoid, phenolic and terpenoid. This research aims to know effectiveness of alpha glucosidase inhibitory effect of ethanol extract of rind, leaf and seed of *p. speciosa* and its correlation to total phenolic content of the extracts. The inhibitory activity of the alpha glucosidase enzyme was measured at λ 405 nm. The result showed that there was correlation between effectiveness of inhibitory and total phenolic content of the extract, that is the higher of the total phenolic content will caused the greater of enzymatic inhibition of extract. The IC_{50} of alpha glucosidase inhibitory effect of ethanol extract of rind, leaf and seed of *P speciosa* are 4,596 ppm, 54,341 ppm, dan 67,425 ppm and the total Phenolic content of the extract are 138,15 mgGAE/g, 59,25 mgGAE/g, dan 36,25 mgGAE/g respectively.

Keywords
Parkia speciosa, Total Phenolic Content, Alpha glukosidase

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1. INTRODUCTION

Diabetes mellitus is a chronic metabolism disorder which characterized by high blood glucose level, caused impaired insulin secretion or insensitivity of cell (Si et al., 2010) . According to (IDF, 2017), diabetes mellitus is including in the 7 to causes of death in the world on 2030.

The raising of DM prevalences, especially Diabetes Mellitus Type 2 (T2D) is a seriously problem. Postprandial hyperglycemia plays an important role in the development of T2D (Telagari and Hullatti, 2015). One of antidiabetic agent for overcome postprandial hyperglycemia is alpha glucosidase inhibitor (Kim et al., 2005). Alpha glucosidase inhibitor act as competitive inhibitors of alpha glucosidase enzyme needed to digest carbohydrate. Inhibition of this enzyme systems helps to reduce the rate of digestion of carbohydrates (Bhat et al., 2011).

Natural product of great structural diversity are a good source for searching for such inhibitors (Qaisar et al., 2014). The petai plant (*Parkia speciosa*) distributed over all on South Sumatera. Traditionally, the seed of the plant used as antidiabetic agent, kidney failure and headache (Azliza et al., 2012). The petai plant (*Parkia speciosa*) have potency as alpha glucosidase inhibitor caused this plant contain flavonoids which spread all over of the plant. The *P. speciosa* seed has a terpenoid compound is lupeol which have anticarcinogen and antiinflamation acivity (Kamisah et al., 2013). Thiazolidyn-4-carbocylic acid and thioprolyn from petai seed has anticarcinogen too (Chen et al., 2008). Methanol extract of petai seed have 2464,3 mgGAE/g total phenolic content with antioxidant activity 5936,9 μmol Troxol/g on DPPH test and 1898,0 μmol Troxol/g on FRAP test (Ali et al., 2011).

The chemical content can be discovered on some place but can be distributed all over of the plant. The rind and leaf of *p. speciosa* suspected have the same chemical content and expected has the same potency as the seed. Phytochemical screening showed that leaf and rind of *p speciosa* contains flavonoid, phenolic and terpenoid. This research aim to know effectiveness of ethanol extract the leaf, rind and seed of p speciosa from South Sumatera as alpha glucosidase inhibitor and its correlation to total phenolic content.

2. EXPERIMENTAL SECTION

2.1 Materials

The *p speciosa* was collected at Ogan Komering Ulu district, South Sumatera. Chemical material for this research are p-nitrophenyl-α-D-glucopiranoside (pNPG), enzyme α-glucosidase and *bovin serum albumin* (BSA) from Sigma-Aldrich, potas-

sium carbonate (Merck®), filter paper , buffer solution pH 6,8, dimetylsulfokside (DMSO) (Merck®), Folin Ciocalteu reagent (Merck®) and Acarbose (Glukobay®)(Bayer Schering Pharma)

2.2 Methods

2.2.1 Preparation of Extract

The material for this research included leaf, seed and rind of p speciosa. Preparation of sample start from washing, drying and powdering. Dried powder (500 g) extracted with 3 L ethanol 96% for 48 h by maceration method. Maceration process repeated for twice. Macerate were filtered through filter paper and concentrated by rotary evaporator.

2.2.2 Inhibitory Activity of Alpha glucosidase Test

Inhibitory activity test was carried out according to standard method by slight modification (Telagari and Hullatti, 2015; Kaskoos, 2013; Najib et al., 2011). Each sample test was determined by adding 55 μL phosphate buffer pH 6,8 and 10 μL substrate PNPG 10 mM, incubated at 37°C for 5 min. Furthermore, 25 μL enzyme solution 0,05 U/ml and incubated for 30 minutes at 37°C. Sodium carbonate 200 mM (100 μL) was added to stop reaction. Absorbance of sample measured using microplate reader at 405 nm. The same procedure was done for control sample but enzyme added after sodium carbonate.

Percentage of inhibitory activity was determined :

$$\%inhibition = \frac{A_0 - A_1}{A_0} x100\% \quad (1)$$

Which, A_0= Absorbance of blank; A_1 = Absorbance of sample

The IC_{50} value calculated by linear regression between sample concentration and percent of inhibition

$$IC_{50} = \frac{50 - a}{b} \quad (2)$$

2.2.3 Determining the Total Phenolic Content (TPC)

The total phenolic content of extract was determined by standard method. The ethanol extract of leaf, seed and rind of *p speciosa* (10 mg) dissolved in 0,5 ml ethanol, diluted with aquadest to 10 ml. This fraction (1ml) added 1,5 ml Folin reagent and shaked and then keep it motionless for 8 minutes. Each of solution added 1,2 ml Na_2CO_3 7,5% and absorbance recorded at 765 nm. The total phenolic content of extract expressed as mg Gallic Acid Equivalent /g extract and calculated by formula:

$$TPC = \frac{c.v.fp}{g} \quad (3)$$

Which, c = Phenolic concentration from linear regression; v = Volume of extract; fp = dilution factor; g = weight of extract

3. RESULTS AND DISCUSSION

3.1 Characterization of extract

Phytochemicals screening was done by standard method on leaf, seed and rind of *p speciosa*. The test was included identification of phenolic, flavonoid and steroid. The result of screening showed at table 1.

Table 1. Phytocemicals screening of *Parkia speciosa* Hassk

Secondary metabolite	Reagent	Rind	Leaf	Seed
Flavonoid	Mg+HCl 2N	+	-	-
	NaOH 2N	+	+	+
Phenolic	$FeCl_3$ 0,1%	+	+	+
Steroid	Liebermen-Buchard	+	+	+

Characterization of extract was done according to standard method (DepKes RI., 2008). The characterization of the extract aims to maintain the consistency and uniformity of the extract quality. Evaluation of characteristic of ethanol extract was done by assessing water content, total ash content, water soluble content, ethanol soluble content. The result for characterization of extract showed in table 2.

The result showed that the seed have the highest of water content. It could be caused by maturation hormone of seed that increase the water content of the seed, whereas in the rind the moisture content is lower because the water content of the rind migrates into the seeds to increase the maturity of the seed (Ridhyanty et al., 2015).

The rind of *p speciosa* has high ash content compared to seeds and leaf. This may be due to the growing sample in the garden close to the smoke of the vehicle causing the rind exposed to smoke of vehicles containing metals. High ash content may be affected by seed variables, growing spots, climate, harvest conditions, post-harvest processing and final preparations such as drying and sieving (Mutiatikum et al., 2010).

The rind and leaf of *p speciosa* have higher content of ethanol soluble than in water soluble, whereas in the seed has the opposite result. This suggests that the content of polar compounds in the seeds is higher than semi polar compounds . The seeds have more polar compound content can be caused due to high carbohydrate levels. This can be linked because water molecules can form hydrates with other molecules containing O and N atoms common to carbohydrates and proteins (FG, 2002).

3.2 Total Phenolic Content

The total phenolic content (TPC) of leaf, seed and rind of *p speciosa* was determined with Folin ciocalteu reagent. Standard solution was gallic acid and absorbance was measured at 765 nm (Paixao et al., 2007). Regression formula obtained y = 0,008x + 0,021 (r = 0,998). The TPC from our research have difference with other researchers (Kamisah et al., 2013). The previous researchers showed that the ethanol extract of leaf have TPC 44,7 mgGAE/g and the seed extract was 51,9 mgGAE/g (Kamisah et al., 2013). Our research showed the TPC of rind, leaf and seed were 138,15; 59,25; 36,25 mgGAE/g respectively. The difference of quality and quantity of plant chemical content could be caused differences of habitats and post harvest processing (Mulia et al., 2016).

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Table 2. Characteristic of *parkia speciosa* extract

Characteristic	Extract		
	Rind	Leaf	Seed
Water content	18,67% ± 3,055	20% ± 0	22,67% ± 2,309
Total Ash Content	20,6%± 0,0005	9,6%± 0,0037	3,3%± 0,0005
Water Soluble Content	15,29%± 0,0234	20,29%± 0,0218	4,6%± 0,0047
Ethanol Soluble Content	22,7%± 0,0233	4,4%± 0,032	21,3%± 0,0288

3.3 Alpha Glucosidase Inhibitory Test

The effectiveness of enzymatic of various extract was determined by calculating IC_{50} 21. Alpha glucosidase inhibitory activity test was done to ethanol extract of leaf, seed and rind of *p speciosa*. Optimum condition for enzyme activity on temperature 37°C , concentration of enzyme 0,05 U/ml, and concentration of substrate 10 mM, pH 6,8 (Najib et al., 2011).

Optimum incubation time for reaction reached by two step. First incubation was 5 minutes at 37°C, it was for reached optimum condition for reaction. The second incubation for 30 minutes was time for bonding between enzyme with all of substrate. Enzyme interaction became effective if pH of solution as same as intestine.

The research showed that the rind of *p speciosa* was the highest inhibition activity with IC50 4,5968. IC_{50} value of each extract and acarbose showed at table 3. IC_{50} value of extract smaller than acarbose, it was indicated that capacity of extract to inhibit of enzyme greater than acarbose.The lower value of IC_{50} showed that the higher enzymatic inhibition (Zhang et al., 2015)

Table 3. The IC_{50} value of Extract and acarbose

Sample	IC_{50}(ppm)	TPC (mgGAE/g)
Rind	4,596	138,15
Leaf	54,341	59,25
Seed	67,425	36,25
Acarbose	162,508	-

Based on TPC and inhibitory activity data, the highest value of test showed by rind of *p speciosa*. There was correlation between TPC and antidiabetic activity. Phenolic compound has capacity as inhibitor of alpha glucosidase activity by bonding with site active of protein (Schafer and P, 2006). Alpha glucosidase inhibitory activity result in postponement of carbohydrate hydrolysis, it was cause reduction of postprandial hyperglycemic (Adisakwattana et al., 2007). Correlation analysis between TPC and IC_{50} value showed that correlation value was -0,998. Coefficient correlation value approach to 1, that mean two variable have good correlation each other and negative value denotes that decrease in the IC_{50} in proportion to the increase in total phenolic content. It can be concluded that TPC of *p speciosa* be responsible to alpha glucosidase inhibitory activity.

4. CONCLUSIONS

Based on research, it can be concluded that IC_{50} value of alpha glucosidase inhibitory activity of rind, leaf and seed were 4,596 ppm, 54,341 ppm and 67,425 ppm. These activity be connected to total phenolic content of extract. Greater of TPC would be more active of extract. Total phenolic content of rind, leaf and seed extract were 138,15 mgGAE/g, 59,25 mgGAE/g and 36,25 mgGAE/g.

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