Inhibition of α-Glucosidase, Total Phenolic Content and Flavonoid Content on Skin Fruit and Flesh Extracts of Some Varieties of Snake Fruits

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Abstract. This study aimed to determine the antidiabetic activity of the skin fruit and flesh of snack fruit through α-glucosidase inhibition and correlated with total phenolic and flavonoid content as well as thin layer chromatography bio-autography. Seven varieties of varieties of skin and flesh of the fruits each extracted by maceration using ethanol 70%. The results show the highest power of the α -glucosidase inhibition obtained at Manonjaya skin extract with IC_{50} value of 17.9 μg/mL. The TLC pattern indicates the presence of four active spot on skin extract and two spots on flesh extracts on the use of solvent BuOH:HAc:water (6:2:2). The highest phenolic content obtained at skin fruit extract of Salak Mawar 186.15 ± 1.66 mg of gallic acid equivalents per gram extract. The highest total flavonoid content obtained in Salak Malaka skin fruit extract that is 7:43 ± 0:04 milli gram of quercetin equivalents

1. Introduction

Controlling blood sugar levels is one way to treat type 2 diabetes mellitus that can delay glucose absorption, ie by inhibiting the enzyme α-glucosidase [1]. This enzyme catalyzes carbohydrates into glucose in the intestinal. Inhibition of α glucosidase can reduce the digestion of complex carbohydrates and absorption of glucose so it can reduce the increasing blood glucose levels.

Indonesia has plants including fruits that can inhibit α-glucosidase. Snack fruits (*Salacca Reinw edulis*) is one of the tropical fruits which are abundant in Indonesia. In Indonesia, almost all parts of snack fruit can be used, such as the fruit used as food, while the seeds can be made into coffee[2]. Normally the skin fruit of snack fruit are not used anymore and became waste, but there are a small percentage of people using the skin fruit and believe it may help diabetic patient. In addition, the flesh fruits also have benefits as an antioxidant. Based on research Leontowicz *et al*. [3] showed that the flesh fruit part of snack fruits of Bangkok, Thailand has high antioxidant capacity. In addition, research of Ariviani and Parnanto [4] showed that the varieties of Salak pondoh, Salak Nglumut, and Salak Bali also has antioxidant capacity.

According Sahputra [5], skin fruit and flesh of snack fruits contain flavonoids, tannins, and alkaloid. The skin fruit and flesh extracts of snack fruits from Yogyakarta do not have α-glucosidase inhibitory activity, while skin fruit extract and flesh extract of snack fruits from Samarinda have inhibitory effect on α-glucosidase. So, it is interesting to find the activity of other variety of snack fruit on α-glucosidase inhibitory. The aims of this study is to determine the α-glucosidase inhibition

activity of skin fruit extract and flesh extract of snack fruits from different varieties, determine the total phenolic and flavonoid content as well as its autobiography thin layer chromatography profile.

2. Materials and Methods

This study is covering sample preparation, extraction by maceration using ethanol 70%, total phenolic and flavonoid content, and profile of active components are determined by TLC.

2.1. Sample Preparation

Sample preparation is including sample collection from Bandung, Tasikmalaya, and Jonggol, West Java, Indonesia. All the snack fruit variety that collected is listed in Table 1. The skin fruit was separated with flesh and seed. The skin fruit and flesh then was drying with oven at 60°C.

Table 1. Snack fruit variety used in this study.

No	Lokal Name	Scientific Name	Collection Place
1	Salak Cililin	*Salacca zalacca* cf Cultivar 'Jawa Lokal'	Bandung, West Java
2	Salak Condet	*Salacca zalacca*	Mekarsari Fruit Park, Jonggol, West Java
3	Salak Malaka	*Salacca zalacca* cf Cultivar 'Manon Jaya'	Bandung, West Java
4	Salak Manonjaya	*Salacca zalacca* cf Cultivar 'Manon Jaya'	Tasikmalaya, West Java
5	Salak Mawar	*Salacca zalacca*	Mekarsari Fruit Park, Jonggol, West Java
6	Salak Pondoh	*Salacca zalacca* cf Cultivar 'Pondoh'	Tasikmalaya, West Java
7	Salak Pondoh Mekarsari	*Salacca zalacca* cf Cultivar 'Pondoh'	Mekarsari Fruit Park, Jonggol, West Java

2.2. Extraction

The dried skin fruit and flesh of snack fruits were extracted with 70% ethanol at 1: 5 by maceration for 24 hours. The extract has been obtained is then evaporated with a rotary evaporator at a temperature of 40-50 ° C in order to obtain the crude extract.

2.3. Total Phenolic Content Determination

Each extract about 25 mg diluted with methanol: water (1: 1) into a 25 ml flask. About 0.9 mL of standard solution (gallic acid) and extract was added by 4.5 mL reagent Folin-Ciocalteau, and shaken with a vortex. After 3 minutes, each solution was added with 3.6 mL of Na_2CO_3 7.5%, shaken and incubated for 1 hour. The absorbance of the standard solution and the sample was measured by UV-Vis spectrophotometer at a wavelength of 765 nm. Total phenol content of samples is determined using a regression equation of standard gallic acid.

2.4. Total Flavonoid Content Determination

Total flavonoid content is determined based on the method of Chang *et al*. [6] with modifications. A total of 10 μL of the extract solution put in a 96 wells microplate, then added with 60 μL ethanol, 10 μL of 10% aluminum chloride, 10 μL of 1M potassium acetate and 120 μL of distilled water. The mixture was incubated for 30 min at room temperature. The absorbance was measured at a wavelength of 415 nm with a microplate reader. Total flavonoid content is reported as Quercetin Equivalent (QE) from the calibration curve of quercetin as standard solution.

2.5. α-Glucosidase Inhibition Test

A total of 10 mL sample solution was added with 50 mL of phosphate buffer pH 7:00 and 25 mL solution of the substrate p-nitrophenyl-α-D-glucopyranose (pNPG), and then incubated at 37 ° C for 5 minutes. The mixture was added with 25 mL of α-glucosidase enzyme solution and incubated for another 15 minutes at 37 ° C. After completion of incubation, the mixture was added 100 mL Na_2CO_3. About 100 μL of the solution is put in a 96 wells microplate and the absorbance of reaction product (p-nitrophenol) was measured at 400 nm.

2.6. Thin Layer Chromatography Profile

Each extract was analysed by Thin Layer Chromatography on silica gel $G_{60}F_{254}$ and BuOH;HAc'water (6:2:2) as eluent. The spots were observed in the UV lamp at 254 and 366 nm. After the finger print of the sample at the two UV wave length were documented, then the TLC plate was sprayed with a solution of α-glucosidase enzyme and incubated for 60 min at room temperature. After incubation the plate sprayed with a solution of p-nitrophenyl α-D-glucopyranose. Spot yellow ribbon will appear after the plate stayed at room temperature. The spot showed that the tested sample is active as an inhibitor of α-glucosidase [7].

3. Results and Discussion

The samples used in the study are seven varieties of snackfruits namely Salak Cililin and Salak Malacca from Bandung, Salak Pondoh and Salak Manonjaya from Tasikmalaya, and Salak Pondoh, Salak Condet, and Salak Mawar from Mekarsari Fruit Garden (Table 1). The physical appearance of whole fruits can be seen in Figure 1. The different physical appearance could result in different chemical component on the flesh and skin fruit of snake fruit. The separated flesh and skin fruit of snack fruits then were extracted by maceration prior to examine the activity as α-glucosidase inhibition. Maceration at room temperature was used in this study because this method is using simple tools and compounds will not damage because it is not high temperature. The yield of extraction is shown in Table 2.

Figure 1. Physical appearance snack fruit used in this study.

The extraction method used to extract the skin and flesh of fruits is maceration. Maceration the extraction process without heating, i.e. immersing the samples using a solvent with stirring performed several times at room temperature. Usually extraction by maceration takes 3 days at the turn of the solvent every day. Extraction using maceration technique has the advantage, using simple tools and samples extracted will be safer because it does not require a high temperature so that the active compound contained in the sample was not damaged.

Based on total phenolic content in Table 2 shows that each extract of the skin fruit and flesh have different total phenolic content. It is influenced by differences in location and the variation of fruits. The skin fruit extract had higher total phenolic content compare with flesh extract. Among the skin fruit, the highest total phenolic content was found in Salak Mawar skin fruit extract, while the lowest total phenolic content was found in Salak Cililin skin fruit extract. Among the flesh extract, Salak Manonjaya had the highest total phenolic content and Salak Cililin had the lowest.

Total flavonoid content of all extract is shown in Table 2. It shows that each extract of the skin fruit and flesh have different total flavonoids content. Total flavonoid content on skin fruits extract is higher compare with the flesh extract. This phenomenon also appears in total phenolic content. It means the

skin fruit is good to be used as source of secondary metabolite such as phenolic and flavonoid. Among the skin fruit, Salak Malaka skin fruit extract had the highest total flavonoids content, while Salak Cililin skin fruit extract had the lowest content. Among the flesh extract, the Salak Manonjaya flesh extract is the highest and the lowest present in Salak Mawar flesh extract.

Table 2. Yield, Phenolic, Flavonoid Contents and IC_{50} value of all extracts.

Snack Fruit Name	Part	Yield (%)	Phenolic content (mg gallic acid equivalen/g extract)	Flavonoid Content (mg quercetin equivalen/g extract)	α-glucosidase IC_{50} (ppm)
Cililin	Skin fruit	8.77	41.90 ± 2.53	2.92 ± 0.06	405.6
	Flesh	79.85	21.05 ± 0.00	0.71 ± 0.18	467.8
Condet	Skin fruit	8.61	141.24 ± 4.07	5.75 ± 0.30	337.5
	Flesh	76.28	21.69 ± 4.66	0.61 ± 0.00	417.9
Malaka	Skin fruit	9.09	132.79 ± 1.92	7.43 ± 0.04	437.5
	Flesh	61.86	61.82 ± 4.50	1.09 ± 0.05	507.1
Manonjaya	Skin fruit	12.12	136.63 ± 2.43	6.45 ± 0.44	17.9
	Flesh	63.92	90.91 ± 4.09	1.24 ± 0.08	502.9
Mawar	Skin fruit	8.92	186.15 ± 1.66	7.01 ± 0.97	27.7
	Flesh	46.98	54.74 ± 1.75	0.46 ± 0.00	274.2
Pondoh	Skin fruit	12.37	138.67 ± 7.23	6.75 ± 0.10	99.1
	Flesh	57.91	65.75 ± 6.37	1.00 ± 0.05	421.7
Pondoh Mekarsari	Skin fruit	10.00	123.85 ± 4.06	4.16 ± 0.20	201.9
	Flesh	93.47	64.99 ± 3.87	0.62 ± 0.00	539.1
Akarbosa (positive control)					6.8

Inhibition of α-glucosidase is expressed in IC_{50}, the concentration of the extract to inhibit 50% activity. The lower IC_{50} value, more potent the extract as α-glucosidase inhibitor. Akarbosa is used as positive control. Based on IC_{50} values in Table 2, the best IC_{50} values for extract obtained in the Salak Manonjaya skin fruit extract, followed by Salak Mawar skin fruit extract. Among the flesh extract, the best α-glucosidase inhibitor is found in Salak Mawar flesh extract. These results suggest that skin fruit extract and flesh extract of Salak Manonjaya and Salak Mawar are potent as α-glucosidase inhibitor. Salak Manonjaya skin fruit extract as the best α-glucosidase inhibitor had the highest phenolic and flavonoids content. Phytochemical compounds in plants have ability to inhibit the α-glucosidase, such as alkaloids, triterpenoids, flavonoids, and also phenolic [8-10].

Thin layer chromatography (TLC) - Bio-autography is a simple and rapid chromatographic method to separate and identify the active compounds. This technique uses direct observations on the spot which is formed after spraying with directly reagents [11]. TLC bio-autography have been developed on inhibiting enzyme activity such as tyrosinase inhibitors, acetylcholinesterase inhibitors, xanthin oxidase inhibitors, and glucosidase inhibitors. TLC bio-autography of α-glucosidase inhibitor is performed by spraying the plate with α-glucosidase enzyme and p-nitrophenyl α-D-glucopyranose as the substrate, the active spot is the spot with yellowish colour on the TLC plate.

The bioautogram of extract is shown in Figure 2 for the skin fruit extract and Figure 3 for the flesh extract. About 4 spots are active from skin fruit extract while on the flesh extract only two active spot. This proves that the skin fruits extract more active in inhibiting the α-glucosidase and also acts as an antidiabetic.

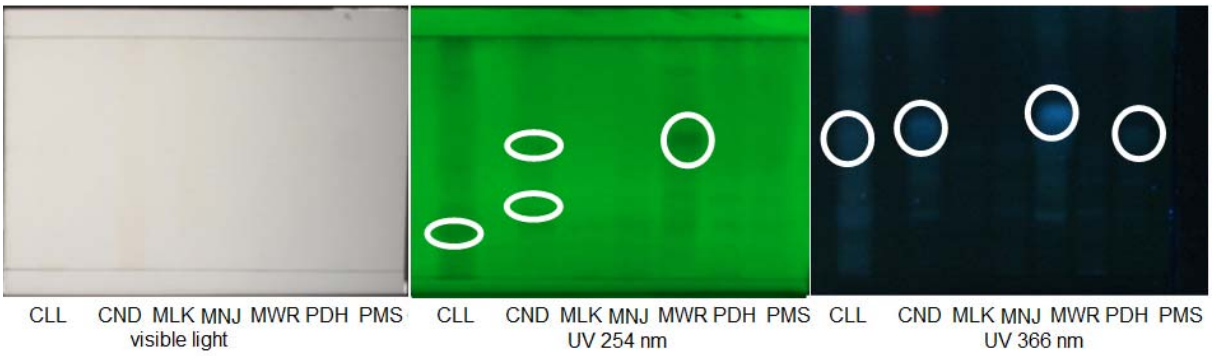

Figure 2. TLC Bioautogram α- Glucosidase of skin fruit extracts of Salak Cililin (CLL), Condet (CND), Malaka (MLK), Mawar (MWR), Pondoh (PDH), Pondoh Mekarsari (PMS).

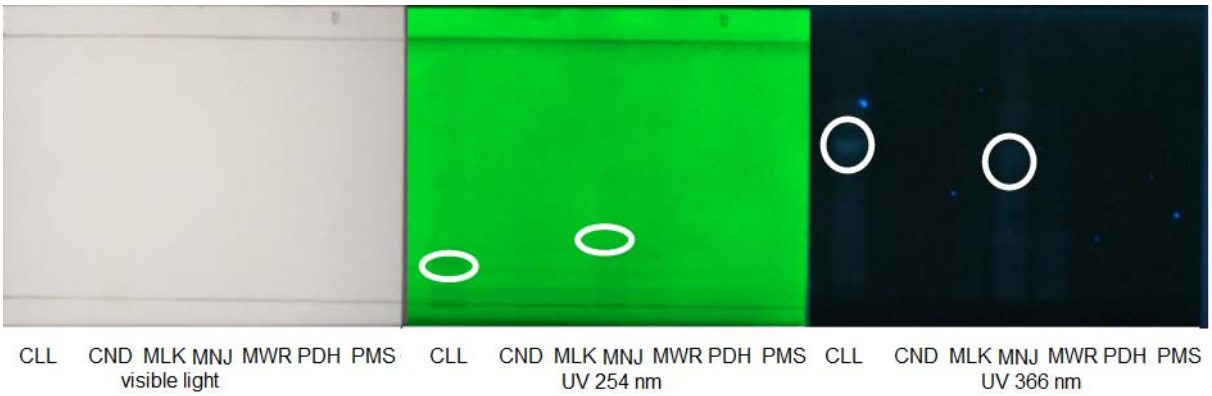

Figure 3. TLC Bioautogram α- Glucosidase of flesh extracts of Salak Cililin (CLL), Condet (CND), Malaka (MLK), Mawar (MWR), Pondoh (PDH), Pondoh Mekarsari (PMS).

4. Conclusion

The highest of total phenolic content of the extract were obtained from the skin fruit of Salak Mawar and the from the flesh of Salak Manonjaya, which each amount are respectively 186.15 and 90.91 equivalent gallic acid/ g extract. The highest of total flavonoid levels were obtained in the extract of skin fruit of Salak Malaka and in the extract of flesh of Salak Manonjaya which each amount are respectively 7.43 and 1.24 mg equivalent quercetin/g extract. The strongest Inhibition of α-glucosidase, stated by the IC50 value, was obtained in the extract of the skin fruits of Salak Manonjaya which amount is 17.9 µg/ mL. Based on the results of the TLC bioautografi inhibitor of α-glucosidase using a solvent which is mixed of butanol: glacial acetic acid: water (6: 2: 2) it was produced active spots for about 4 spots on the extract of skin fruits and another 2 spots on the extract of flesh.

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