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Potential Antioxidant and Antidiabetic Activities of Kayu Kuning (*Arcangelisia flava*)

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Abstract

Kayu kuning has already used as traditional medicine to treat various disease. The aim of this study was to evaluate the in vitro antioxidant and antidiabetic activity of hexane, ethyl acetate, and methanol extract of Kayu kuning leaves. The antioxidant activities of the Kayu kuning extracts were measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH), superoxide radical, and hydroxyl radical assays. The antidiabetic activity was evaluated by α-amylase and α-glucosidase inhibition assays. Compared with the standard vitamin C, extract hexane, ethyl acetate, and methanol of Kayu kuning shown potent as antioxidant. The methanol extract showed highest scavenging activity on superoxide and hydroxyl radical. The ethyl acetate extract showed highest scavenging activity on DPPH radical. Compared with the standard acarbose, extract hexane and ethyl acetate of Kayu kuning shown potent as antidiabetic while the methanol extract had no potent. The ethyl acetate extract showed highest potent as antidiabetic.

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Keywords: antidiabetic, antioxidant, Kayu kuning, scavenging activity, inhibition assay

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1. Introduction

The pattern of disease at this time began to shift from infectious diseases to the metabolic syndrome. Diabetes mellitus is one of metabolic syndrome that is highly prevalent globally. The global prevalence of diabetes mellitus for population ages 20 to 79 years was 149 million (5.1%) in 2003 and was estimated to reach 333 million (6.3%) in 2025 (International Diabetes Federation, 2003)

Diabetes mellitus disease is characterized by the accumulation of blood glucose exceeds the normal condition (hyperglycemia). Blood glucose can undergo reactions autoxidation, which catalyzed by transition metal groups (Wolff, 1993). Autoxidation reaction product from glucose is reactive oxygen species such as superoxide anion radical, hydroxyl radical and hydrogen peroxide (Wolff, 1993). The increasing of free radicals in the body stimulates oxidative stress when the antioxidants in the body are unable to overcome free radicals. The result is damage of cell organelles, the increasing in lipid peroxidation, and improving insulin resistance. This causes the appearance of various complications (Maritim et al., 2003).

Antioxidant and antidiabetic pharmacological therapy uses synthetic drugs such as acarbose and butyl hydroxyanisole (BHA) leading a negative effect. The negative effect from acarbose in gastrointestinal is bloating, nausea, diarrhea, and flatulence (Sudha et al., 2011) while the BHA is toxicological and carcinogenic (Nanheesha et al., 2007). This condition causes a great deal of research to find natural drugs derived from plants.

Kayu kuning is one of the medicinal plants that growth in the Meru Betiri National Park. Kayu kuning has used as a traditional medicine to treat malaria, dysentery, and fever in Kalimantan (Subeki et al., 2004). Isolation of tryacontanyl caffeat from Kayu kuning trunk shown potential as an antioxidant (Keawpradub et al., 2005). The potential of Kayu kuning in treating these diseases may be caused by the content of phenolic compunds. Therefore, in this research conducted to exploration potential of phenolic extract from Kayu kuning leaves as an antioxidant and antidiabetic. The potential phenolic extracts as antioxidants was seen from its ability to scavenging the activity of free radicals, while its potential as an antidiabetic through inhibition of enzyme activity. Free radicals were analyzed DPPH, superoxide anion, and hydroxyl while the enzymes were α-amylase and α-glucosidase.

2. Materials and Methods

2.1 Chemicals and reagents

Kayu kuning leaves; hexane (Merck); ethyl acetate (Merck); methanol (Merck); distilled water; Folin-Ciocalteu reagent (Merck); sodium carbonate (Merck); gallic acid (Sigma-Aldrich); sodium nitrite (Merck); aluminum(III) chloride (Merck); sodium hydroxide (Merck); quercetin (Nacalai tasque); formic acid (Merck); 1,1-diphenyl-2-picryl-hydrazil (Nacalai tasque); ethanol (Merck); vitamin C (Nacalai tasque); ethylenediaminetetraacetic acid (EDTA) (Nacalai tasque); iron(III) chloride (Merck); hydrogen peroxide (Sigma-Aldrich); 2-deoxy-D-ribose (Sigma-Aldrich); potassium dihydrogenphosphat (Merck); trichloroacetic acid (Merck); 2-thiobarbituric acid (Sigma-Aldrich); pyrogallol (Sigma-Aldrich); trizma-base (Nacalai tasque); hydrochloric acid (Merck); sucrose (Merck); dimethylsulfoxide (Merck); sodium hydrogenphosphat (Nacalai tasque); peroxidase (Sigma-Aldrich); α-glucosidase (Sigma-Aldrich); Glucose oxidase (Sigma-Aldrich); 4-aminoanipyrine (Sigma-Aldrich); phenol (Sigma-Aldrich); sodium chloride (Merck); potassium sodium tartrate (Merck), soluble starch (Merck), 3,5-dinitrosalicyilic acid (Merck), α-amylase (Sigma-Aldrich), potassium hydrogenphosphate (Merck), triton X-100 (Sigma-Aldrich), and acarbose (Glucobay).

2.2 Maceration of Kayu kuning leaves

Kayu kuning plant was collected from Meru Betiri National Park, Jember, Indonesia. The taxonomical identity of the plant was confirmed by Institutions Meru Betiri National Park. Maceration was done in three stages with different polarity of solvent. They were hexane, ethyl acetate, and methanol. Maceration started from nonpolar solvent hexane, followed by ethyl acetate and ended with methanol. 50 mg simplisia of Kayu kuning leaves was macerated with 250 ml solvent. The time incubation of each solvent was 72 hours. Three types of extracts were

produced from this step. They were hexane Kayu kuning (HKK), ethyl acetate Kayu kuning (EKK), and methanol Kayu kuning (MKK).

2.3 Total phenolics assay

The total phenolics were determined by the Folin-Ciocalteu method based on standard gallic acid (Taga et al., 1984). Briefly, 50 µl of the sample extracts were transferred into test tube and mixed with 1 ml Na_2CO_3 2% (w/v). After 2 min, 50 µl of Folin-Ciocalteu reagent 50% (v/v) was added and allowed to stand for 30 min. The asorbance of the solution was read by UV-Vis spectrophotometer at 750 nm. The standard curve was estabilished using various concentrations of gallic acid, and results were expressed as mg of gallic acid equivalent (GAE) per gram of sample extract.

2.4 Total flavonoids assay

The total flavonoids were determined by the AlCl3 10% method based on standard quercetin (Chang et al., 2002). Firstly, 150 µl of the sample extracts were transferred into test tube and it was added with 400 µl of distilled water. This solution was added with 30 µl of NaNO2 5% (w/v) and allowed to stand for 5 min. Secondly, 30 µl of the AlCl3 10% (w/v) wes added to the test tube, shaken, and was left to stand for 6 min. Finally, 200 µl of NaOH 1 M and 240 µl of distilled water was added, shaken, and the asorbance of the solution was read at 415 nm. The standard curve was estabilished using various concentrations of quercetin, and results were expressed as mg of quercetin equivalent (QE) per gram of sample extract.

2.5 Thin Layer Chromatography (TLC) assay

TLC was used to separate the chemical contituens of Kayu kuning extracts using the DPPH as visualizer (Wang et al., 2012). The method employed silica gel 60 F254 as stationary phase and mixture of ethyl acetate-formic acid-water (82:9:9, v/v/v) as mobile phase. Samples (3,5 µl; equivalent to 1,75 mg GAE) were spotted in the plate. The plate was developed to distance 8 cm in the chamber. After development, the plate was sprayed with DPPH 0,3 % to produce the colour. Methanol was used as negative control.

2.6 In vitro testing of Kayu kuning extracts antioxidant activity

2.6.1 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH scavenging activity was determined by UV-Vis spectrophotometry method (Soler-Rivas et al., 2000). Two hundred microliters of the extracts with different concentrations was transferred into 96-well plates. One hundred microliters of 90 µM DPPH reagent was added to 96-well plates. The extract was replaced with solvent for control. After 30 min, reaction reduction of DPPH was measured at an absorbance 515 nm. Vitamin C was used as standard for this assay. Percentage inhibition was calculated using the formula:

$$\%\,\text{Inhibition} = \left[\frac{\left(A_o - A_1\right)}{A_0}\right] \text{x}\,100$$

A_0 were control absorbance and A_1 were absorbance of the extract.

2.6.2 Scavenging activity of the superoxide anion assay

The superoxide anion scavenging activity was determined by UV-Vis spectrophotometry method (Tang et al., 2010). Two hundred microliters of sample extracts was added to 1.7 ml of buffer 50 mM Tris-HCl (pH 8.2) in the test tube. The mixture was incubated at room temperature for 10 min, and then 100 µl of 10 mM pyrogallol (in HCl 10 mM) was added. The absorbance of the reaction mixture was measured at 320 nm for 4 min reaction. The same concentration of pyrogallol without sample extract was used as positive control. Vitamin C was used as standard for this assay. Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \left[\frac{(S_o - S_1)}{S_0} \right] x\,100$$

S_0 were control slope and S_1 were slope of the extract. This analysis used vitamin C as standard.

2.6.3 Scavenging activity of the hydroxyl assay

The hydroxyl scavenging activity was determined by the ability of the different extracts of Kayu kuning to scavange the hyroxyl radicals generated by Fenton reaction (Halliwell et al., 1987). The reaction mixture inn final volume 0f 500 µl containded 50 µl 2-deoxy-D-ribose 28 mM (in 20 mM phosphate buffer; pH 7.4), 150 µl of extracts, 100 µl of EDTA 1 mM, 100 µl of $FeCl_3$ 10 mM, 50 µl of H_2O_2 1 mM, and 50 µl of vitamin C 1 mM. This solutions were kept at 37° C for 1 h. Five hundred microliters of 2-thiobarbituric acid 1% and 500 µl of trichloroacetic acid 2.8% was added. The solution incubated again for 20 minutes at 100° C to produce a pink colour. After cooling, the absorbance was measured by UV-Vis spectrophotometer at 532 nm against a blank containing 2-deoxy-D-ribose and buffer. Vitamin C was used as standard for this assay.

2.7 In vitro testing of Kayu kuning extracts antidiabetic activity

2.7.1 α-Amylase inhibition assay

Inhibition of α-amylase by Kayu kuning leaves extracts were analyzed by UV-Vis spectrophotometry method (Hashim et al., 2013) with slightly modified. Briefly, porcine pancreatic α-amylase was dissolved in phosphate buffer (20 mM), pH 6,9. Four test tubes were prepared. One hundred microliters of the extracts transferred into the microtube. One hundred of DMSO solution was used as a control positive and it transferred to another microtube. 150 µl of α-amylase (0.1 units/ml) was added to the microtube containing the extract and DMSO, while the sample control and blank control was made by replacing the α-amylase with phosphate buffer pH 6.9. The solution was preincubated for 15 minutes at 37°C after it was shaked. After it, 250 µl of soluble starch (1% w/v) was added into all microtube. The solution was incubated for 15 min at 37 °C after it was shaked. Hydrolysis reaction was stopped by boiling for 1 min and from each microtube was taken 160 µl to be transferred into other microtubes. The solution was boiled for 15 min after added 80 µl DNS reagent to determine the total reducing sugar. After it was diluted with 720 µl of distilled water and from each microtube was taken 200 µl to be transferred into 96-well plates, the absorbance was measured with microplate readers at 540 nm. Acarbose was already marketed antidiabetic used as standard. Percentage inhibition of α-amylase by each extract was calculated by the following formula:

$$\% \text{ Inhibition} = \left[\frac{\left[\left(C^+ - C^- \right) - \left(S^+ - S^- \right) \right]}{\left(C^+ - C^- \right)} \right] x\,100$$

C^+ was a sample control with enzyme and C^- was the sample control without enzyme, while the S^+ was a sample with enzyme and S^- was a sample without enzyme.

2.7.2 α-Glucosidase inhibition assay

Inhibition of α-glucosidase by Kayu kuning leaves extracts were analyzed by UV-Vis spectrophotometry method (Miyazawa et al., 2005) with slightly modified. Four test tubes ware prepared. One hundred microliters of the maltose 0,125 M transferred into each microtube. One hundred microliters of the extracts was added to the two microtubes. One hundred microliters of the DMSO solution was added to the other microtubes. After it was added 190 µl of phosphate buffer pH 7 into each microtube. The solution was vortexed and it added 10 µl of α-glucosidase (1 unit/ml) to one microtube containing extract and one containing DMSO. Aquabides was used as the control by replace α-glucosidase to other microtubes. The solution was incubated at 37° C for 1 ho after it was vortexed. The reaction was stopped by boiling the solution for 3 min. The solution from each tube was taken of 235 µl to transferred to other microtubes. After it, 750 µl of phenol buffer pH 7 was added to each microtube. Than it was coupled with 5 µl of peroxidase (0.5 unit/ml), 5 µl of aminoantipyrine, and 5 µl of glucose oxidase (0.8 unit/ml).

After the solution was vorrtexed and incubated again for 10 minutes at 37° C. It was pipetted 200 µl and transferred into 96-well plates to measure the absorbance at 500 nm with microplate readers. Percentage inhibition of α-glucosidase by each extract was calculated by same formula for calculated α-amylase inhibition. Acarbose was used as standard for this assay.

3. Results and Discussion

The maceration yield, total phenolic content, and total flavonoid of Kayu kuning leves extract is presented in Table 1. The maceration with methanol gave the highest yield (17,0%), whereas macration with hexane gave the lowest yield (2,76%). Methanol could dissolve the phenolic compounds that were polar to nonpolar. It caused methanol have the highest yield.

It was known that phenolic compounds, especially flavonoid compounds are responsible for effective free radical scavenging. Total phenolics and total flavonoids in the various extracts of Kayu kuning were also determined and found to be in following decreasing order: methanol > ethyl acetate > hexane. From the data, it is evident that methanol extract has higher phenolic content (135,25±6,02 mg GAE/g) and flavonoid content (280,61±2,01 mg QE/g).

Table 1. Maceration yield, total phenolics, flavonoids content of Kayu kuning leaves extract

Extract	Maceration yield (%)	Total Phenolics (mg GAE/g)	Total Flavonoids (mg QE/g)
HKK	2,76	23,36±0,68	59,45±3,96
EKK	4,41	114,99±4,83	147,89±3,77
MKK	17,00	135,25±6,02	280,61±2,01

Each value represents the mean ± SD, except % yield.

3.1 Thin Layer Chromatography

To screen the phenolic compounds from various extracts of Kayu kuning leaves, a TLC method was performed. After separation on TLC plate, the compounds with radical scavenging activity were determined with DPPH reagent. The TLC plate produced different colour depending on chemical compounds on each spot (Figure 1).

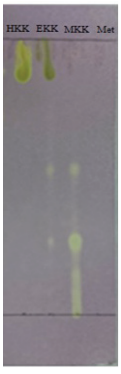

Figure 1. The chromatograms of phenolic extracts from Kayu kuning

As shown in Figure 1, the samples produced yellowish spots on the purple background were considered as phenolic compounds. The background color of the plate changed from purple to yellowish after DPPH radical

reduced by phenolic compound. Methanol extract of Kayu kuning had the highest number of spots. Some of the spots that appeared in the extract of hexane and ethyl acetate also appeared in the methanol extract. It showed that the methanol solvent capable to dissolving the phenolic compounds with various polarity.

3.2 Antioxidant activities of the Kayu kuning leaves extract

Quantitative phytochemical analysis indicated that methanol extract has the highest phenolics and flavonoids content. These classes of compounds were responsible for free radical scavenging effect. The determination of antioxidant activity of Kayu kuning extracts used various radicals give different results (Table 2). The results showed that methanol extract exhibited the strongest DPPH radical scavenging ability (79,68±0,17%) and hydroxyl radical scavenging ability (90,51±0,08%). The highest quantities of flavonoid contents in methanol extract may be responsible for its free radical scavenging activity. Ethyl acetate extract showed exhibited the strongest superoxide anion radical scavenging ability (22,16±0,73%). Ethyl acetate extract had medium quantities of flavonoid contents. All of assays were done in the same concentration of phenolic compounds in the extract. The concentration phenolic was used to assay antioxidant activity of extract was 2 µg GAE/ml.

Table 2. Percentage of DPPH, superoxide, and hydroxyl radical scavenging effect

Extract	% of inhibition		
	DPPH radical	Superoxide radical	Hydroxyl radical
HKK	55,14±0,20	16,74±0,04	59,25±0,25
EKK	64,50±0,48	22,16±0,73	73,07±0,96
MKK	79,68±0,17	12,17±0,11	90,51±0,08
Vitamin C	66,99±0,72	20,43±0,57	88,78±0,08

Each value represents the mean ± SD.

3.3 Antidiabetic activities of the Kayu kuning leaves extract

In this study, α-amylase inhibitory activity was evaluated against the enzyme obtained from mammal (porcine pancreatic). α-Glucosidase was obtained from yeast (*S. cerevisiae*). Acarbose was used as standar. The results showed that the ethyl acetate extract has the highest percentage of α-amylase inhibition (64,24±3,53%) but its ability still lower than acarbose. The methanol extract has the highest percentage of α-glucosidase inhibition (95,04±3,55%) and its ability more than acarbose.

Table 3. Percentage Inhibition of α-amylase and α-glucosidase

Extract	% of inhibition	
	α-amylase	α-glucosidase
HKK	43,18±0,79	61,23±3,20
EKK	64,24±3,53	78,96±2,28
MKK	3,48±1,60	95,04±3,55
Acarbose	91,06±2,15	54,85±1,48

Each value represents the mean ± SD.

The highest quantities of flavonoid contents in methanol extract may be responsible for its α-glucosidase inhibition activity. All of assays were done in the same concentration of phenolic compounds in the extract. The

concentration phenolic was used to assay antidiabetic activity of extract was 25 μg GAE/ml. Ethyl acetate extract has the highest potential as antidiabetic because it has a high inhibitory activity on α-amylase and α-glucosidase. Table 3 showed percentage inhibition of α-amylase and α-glucosidase by Kayu kuning leave extracts. Methanol extract did not chosen as highest potential as antidiabetic because it has very low percentage inhibition of α-amylase.

4. Conclusion

From the above study, it can be concluded that phenolic extract of Kayu kuning leaves posseses the potential as antioxidant activity which is comparable that of the standard vitamin C. The methanol extract exhibited the strongest DPPH radical scavenging ability and hydroxyl radical scavenging ability. The ethyl acetate extract exhibited the strongest superoxide anion radical scavenging ability. The ethyl acetate and hexane extract of Kayu kuning leaves posseses the potential as antidiabetic activity which is comparable that of the standard acarbose. The ethyl acetate extract posseses the most potential as an antidiabetic because it has a high inhibitory activity on α-amylase and α-glucosidase.

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