Antioxidant Activity and Antocyanin Derivatives Study of Red *Katuk* (*Euphorbia cotinifolia* L.) Leaves

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Abstract

In this study, antioxidant activity and antocyanin derivatives study of extract of red *katuk* (*Euphorbia cotinifolia* L.) leaves was done. Antioxidant activity was done by DPPH (*2,2-diphenyl 1-picrylhydrazyl*) method using ultraviolet-visible spectrophotometer and thin layer chromatography. The result showed that methanol extract had antioxidant activity with IC_{50} value 33.13 µg/mL. Vitamin C as comparative substance had IC_{50} value was 5.24 µg/mL. From the acidified methanol extract, two anthocyanin derivatives have been isolated by preparative paper chromatography and characterized by ultraviolet visible spectrophotometry, which were supposed to be peonidin and cyanidin.

Keywords: *Euphorbia cotinifolia* L, antioxidant, antocyanin

Abstrak

Pada penelitian ini dilakukan pengujian mengenai aktifitas antioksidan dan turunan antosianin dari ekstrak daun katuk (merah *Euphorbia cotinifolia* L.). Aktifitas antioksidan dilakukan dengan metode DPPH (*2,2-diphenyl 1-picrylhydrazyl*) menggunakan spektrofotometer UV-Vis dan kromatografi lapis tipis (KLT). Hasil penellitian menunjukkan bahwa ekstrak metanol memiliki aktifitas antioksidan dengan nilai IC_{50} 33,13 µg/mL. Vitamin C sebagai senyawa pembanding memiliki nilai IC_{50} 5,24 µg/mL. Dari ekstrak metanol dalam suasana asam, terdapat 2 turunan antosianin yang berhasil diisolasi menggunakan kromatografi kertas preparatif dan dikarakterisasi dengan spektrofotometer UV-Vis, dimana kedua isolate tersebut diketahui sebagai peonidin dan sianidin.

Kata kunci : *Euphorbia cotinifolia* L, antioksidan, antosianin

Introduction

Red *katuk* (*Euphorbia cotinifolia L.*) is one of traditional medicinal plants which some Garut communities using the cooking water of that leaves in treatment of diabetic. Red *katuk* plant originated from tropic America. The leaves are oval and purple. This plant is a shrub or small tree. This plant can grow in various places, especially in cold areas including some Garut territorial (Jones and Arlene 1987; Putri 2005).

Antocyanin is one of many leaf pigments which found in some plants, especially red plants. According to Harborne (1987), antocyanins in weak acid chloride solution have obtained one peak with spesific wavelengh areas if characterized by ultraviolet-visible spectrophotometer.

The majority of the antioxidant activity from plants is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Khalaf *et al*. 2008). It has been investigated that red *katuk* leaves contain flavonoid and phenolic acids (Ardiansyah 2005).

The investigation on antocyanin and antioxidant activity of red *katuk* leaves has not been done. On behalf of that, this research would be done those investigations.

Experiments

Materials

Red *katuk* (*Euphorbia cotinifolia L.*) herbs (The determination of taxonomic plant had been done at the Bandungense Herbarium, School of Biology, Institut Teknologi Bandung), distilled water, methanol, DPPH (*2,2-diphenyl-1-picrylhydrazyl*) (Sigma Aldrich), chloride acid, sulphuric acid and the others material.

Equipments

Dried oven, grinding apparatus, microscope, maceration apparatus, distillation apparatus, chromatography chamber, rotary evaporator, ultraviolet lamp, ultraviolet spectrophotometer, micropipete and the others equipment.

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Procedures

The Study of Antocyanin Derivatives of Red *Katuk* Leaves

Fresh red *katuk* leaves were heated in 2 M of HCl (100°C) for 4 minutes. The filtrate (antocyanin extract) was rinsed twice with ethyl acetate. The water layer was heated (80°C) for 3 minutes, then extracted by amyl alcohol. The amyl alcohol layer was evaporated, then diluted in 2 N HCl in methanol, it obtained pigment solution which analyzed by paper chromatography and separated by preparative paper chromatography. The study of antocyanin derivatives method of red *katuk* leaves is presented in Figure 1.

The antioxidants activity of red *katuk* (*Euphorbia cotinifolia* L.) leaves

About 100 g of dried, ground plant materials were macerated in methanol for 24 hours. The macerated materials was stirred several times. Those procedure was repeated for 3 times. The final extracts were passed through filter paper, it obtained 6.7 litre. The filtrats were concentrated under vacum on a rotary evaporator. The monitoring of methanol extract was done by thin layer chromatography using visible, 10% sulphuric acid in methanol and 0.2% DPPH in methanol as spray reagents. The antioxidant activity method of red *katuk* leaves is presented in Figure 2.

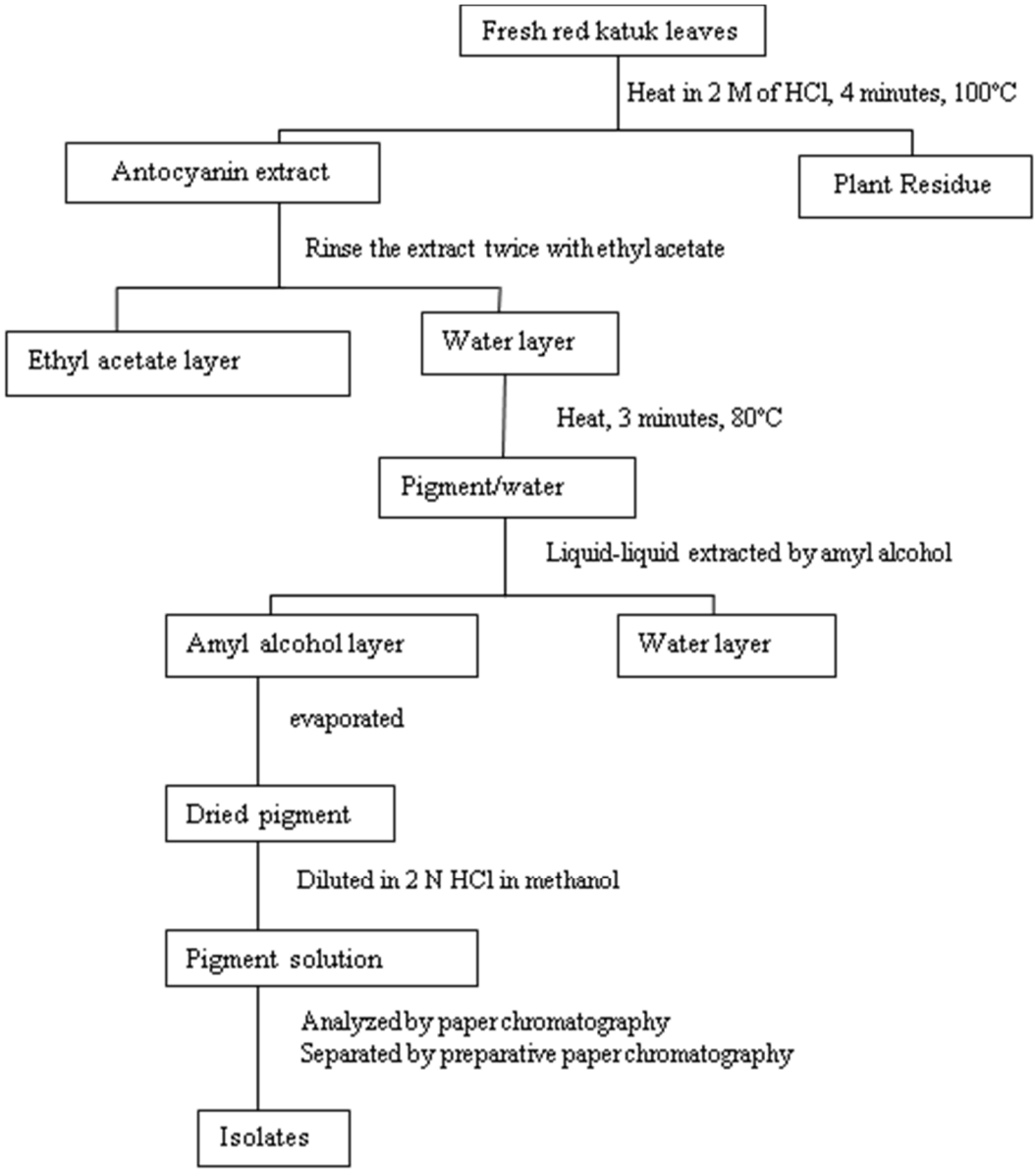

Figure 1. Antocyanine derivatives study scheme

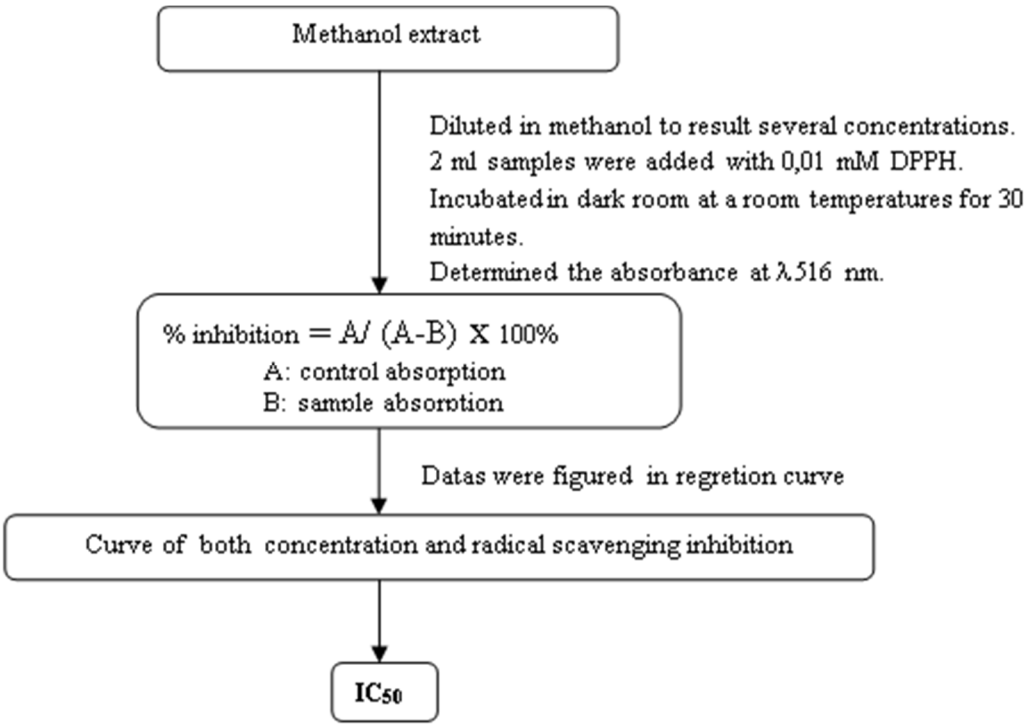

Figure 2. Antioxidant activity scheme

Result and Discussion

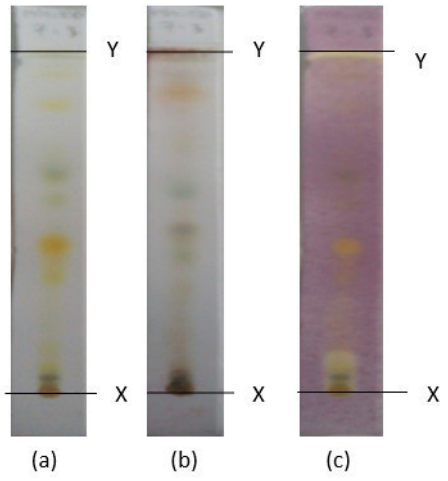

Figure 3. The result of thin layer chromatography. (a) visible; (b) 10% of sulphuric acid in methanol; (c) 0.2% of DPPH in methanol. X = spotted line, Y = developer line. Stationary phase: silica gel $GF_{254,}$ Developer: n-hexane-aethyl acetate (7 : 3).

The analytical paper chromatogram (using two mobile phase solution) of the pigment solution (yielded by Figure 1) presented at Figure 4. The separation of pigment solution has been done by preparative paper chromatography to obtain four isolates. Isolate A and isolate B are obtained from the first developer solvent (BAW: 4:1:5 upper layer), whereas isolate C and isolate D are obtained from the second developer solvent (Forestal: 3:30:10).

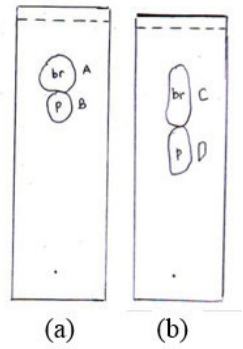

Figure 4. Paper chromatogram of pigment solution. (a) paper chromatogram of pigment solution using BAW (4:1:5) as a developer solvent; (b) paper chromatogram of pigment solution using Forestal (3:30:10) as a developer solvent. Reagent spray: 5 % of $AlCl_{3.}$ br: brown; p: pink.

The purity of isolate B has given hRf 73.19 and violet spot (using 5% $AlCl_3$ as a reagent spray). Isolate B has been done purity by two dimensional paper chromatography and characterized by UV-visible spectrophotometry (using 2 N HCl in methanol as a

standard solution). Isolate B has given one peak (533 nm, 0.3 A), which according to Harborne (1987) (532 nm, hRf 71), it was supposed to be peonidin.

Isolate D has given hRf 50.4 and pink spot (using 5% $AlCl_3$ as a reagent spray). The purity has been tested by two dimensional paper chromatography and also characterized by UV-visible spectrophotometry. The purity of isolate D has given one peak (535 nm, 0.8 A), which according to Harborne (1987) (535 nm, 49 hRf), so it was supposed to be cyanidin.

DPPH has been used for antioxidant activity test because it is simple, easy, and fast. Antioxidant compounds reacts with DPPH radical by hidrogen atomic donation mechanism and eliminated DPPH's colour. This study used 516 nm as a maximum wavelengh. DPPH must be stored in dark place and avoided from light. Free radical scavenging activity is signed by colour changing (purple to yellow).The strong of free radical scavenging activity could be known by compared it with standard antioxidant, which has established of antioxidant activity. This study was used vitamin C, as a standard antioxidant. Parametric value that has been used this method was IC_{50}.

Table 1. *In Vitro* Antioxidant Activity of Red *Katuk* (*Euphorbia cotinifolia* L.) Leaves Extract

No.	Concentration (µg/ml)	Absorbance ± SD	Inhibition (%)
	Control	0.7618	
1.	1	0.6633±0.0049	12.93
2.	2.5	0.6228±0.0034	18.25
3.	5	0.5956±0.0013	21.82
4.	10	0.5902±0.0024	22.52
5.	25	0.4442±0.0009	41.69
6.	50	0.2415±0.0006	68.30

Table 2. *In vitro* antioxidant activity of vitamin C

No.	Concentration (µg/ml)	Absorbance ± SD	Inhibition (%)
	Control	0.7033	
1.	1	0.4535±0.0060	35.52
2.	2	0.4271±0.0065	39.27
3.	3	0.3965±0.0062	43.62
4.	4	0.3638±0.0055	48.27
5.	5	0.3548±0.0021	49.55
6.	6	0.3474±0.0041	50.60

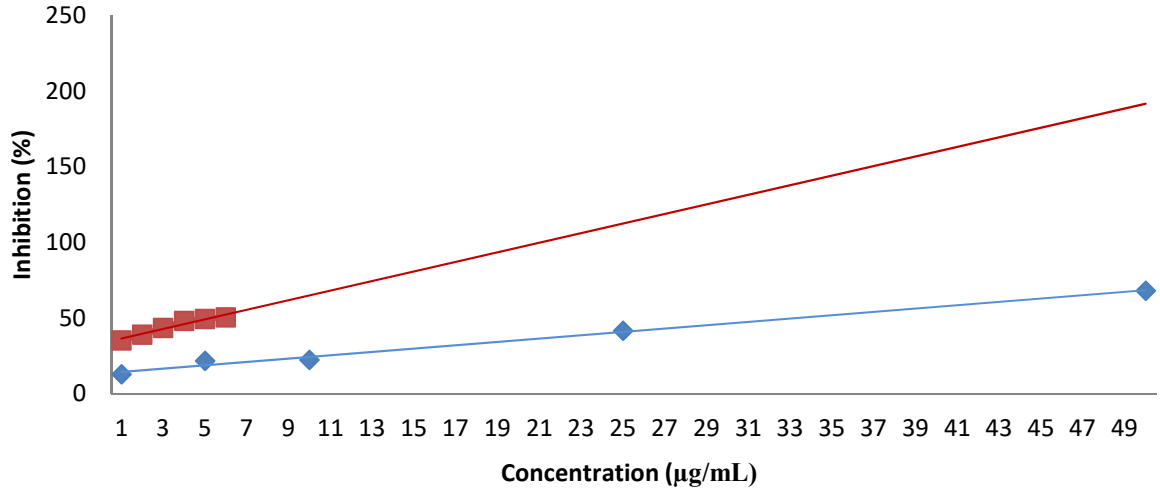

Figure 5. DPPH free radical scavenging activity of standard ascorbic acid and red katuk (*Euphorbia cotinifolia L.*) leaves extract. Red *katuk*; Vitamin C.

Conclusion

From the acidified methanolic extract, two anthocyanin derivatives have been isolated by preparative paper chromatography and characterized by uv-vis spectrophotometry, which were supposed to be peonidin and cyanidin. From the antioxidant test of red *katuk* leaves using DPPH (*2,2-diphenyl-1-picrylhydrazyl*), it showed that methanol extract had antioxidant activity with IC_{50} value was 33.13 µg/mL. Vitamin C as a comparative substance had IC_{50} value was 5.24 µg/mL.

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