

The Study of Antioxidant and Antityrosinase Activity of Extract from Mulberry Root (*Morus alba* L.)

Faizatun^a, e. Anwar^b, j. Djajadisara^b, e. Mardliyati^c

^aFaculty of pharmacy, Pancasila University, Indonesia

^bFaculty of pharmacy, University of Indonesia, Indonesia

^cPharmaceutical and Medical Technology Center, BPPT, Indonesia

Abstract

Objective: The objective of this study was to investigate the antioxidant and antityrosinase activities of the extract of mulberry root related to oxyresveratrol content and to find out that which kind of solvent giving the highest antioxidant and antityrosinase activities.

Methods: Powdered mulberry root passed through a 40-mesh sieve and extracted by maceration with ultrasonic using ethanol and methanol into various portions. Extraction solvents were used ethanol 50% (E50), 70% (E70), 96% (E96) and methanol 60% (M60), 85% (M85), 100% (M100). All of the extracts were tested antioxidant activities by DPPH method and antityrosinase activities. High-performance liquid chromatography (HPLC) analysis was employed to determine the oxyresveratrol content in all of the extract mulberry root.

Results: The levels of the oxyresveratrol varied in different portions of the varieties of solvents. The amount of oxyresveratrol was very low in all samples, 0.85 – 2.17%. Oxyresveratrol levels were the highest in the M85 extract. TLC identification of compounds was fluorescent under UV 254 and 366 nm, spotting of E96 and M100 of the mulberry root extract had fluorescence which was stronger than the other mulberry root extract. All extract might serve as a natural antioxidant and tyrosinase inhibitor. The E96 extract exhibited the highest radical scavenging and tyrosinase inhibitory activity than the extract with another solvent.

Conclusion: Antityrosinase and antioxidant activity of 96% ethanol extract of mulberry root were stronger than the five other extracts, not related to oxyresveratrol content in extracts. Oxyresveratrol levels was the most highest in the methanol extract 100%.

Keywords: antioxidant, antityrosinase, *Morus alba*, oxyresveratrol,.

INTRODUCTION

Mulberry contains phenolic compounds including stilbene groups which can inhibit enzyme tyrosinase activity and as an antioxidant, so its potential in cosmetic [1,2]. Oxyresveratrol was an aglycone of mulberroside A and showed strong inhibit enzyme tyrosinase activity [3]. Zhou Jin et al, 60% methanol extract of mulberry root contains constituent highest compared to with other concentration of ethanol and methanol. In addition, the mulberry root extract obtained oxyresveratrol amount to 374.6 ± 4.1 $\mu\text{g/g}$ more higher than oxyresveratrol of mulberry leaf extract 144.5 ± 1.6 $\mu\text{g/g}$ [3,4]. In study Jeong Keun Kim et al, oxyresveratrol of mulberry root extract ethanol at

concentration of 2.5 μM almost completely inhibit tyrosinase activity [5]. In study, Lee Hee Sang et al, performed using solvent extraction with 85% methanol to produce mulberry root extract with IC_{50} value of 68.3 $\mu\text{g/ml}$ [6].

Therefore, in this study conducted extraction of mulberry root bulbs that grow in Bogor, Indonesia by using 50% ethanol (E50), 70% ethanol (E70), 96% ethanol (E96), 60% methanol (M60), 85% methanol (M85) and 100% methanol (M100). Extraction methods was maceration combination with sonication. The extract obtained would be compared to the amount of yield, TLC separation, the activity of the enzyme tyrosinase inhibition, in vitro antioxidant activity

with DPPH (free radical reduction) methods and assay oxyresveratrol in crude extracts.

MATERIALS AND METHODS

Materials

Plant has been procured from *Rumah Sutura*, Bogor, Indonesia and it has been authenticated by Research Center for Biology, Indonesian Institute of Sciences, Indonesia, with the Voucher Specimen No. 1048/IPH.1.01/If.8/VII/2015. Reference standard oxyresveratrol (Sigma), Enzym tyrosinase (Sigma), L-Dopa (Sigma), DPPH, ethanol, kojic acid, methanol, ascorbic acid, Silica gel 60 F254 plates, acetonitrile, formic acid.

Preparations of extracts

Each of 300 g powder of root was extracted by maceration combination with sonication in ethanol and methanol in various portion. They were soaked at room temperature for 24 hrs and sonicated for 40 minutes. Each extract was evaporated under vacuum until dried. The percentage yield of each crude extract was calculated. All crude extracts were kept in refrigerator at 4°C.

Determination of antioxidant activities with DPPH radical-scavenging method

The stable free radical DPPH (DPPH) reacted with antioxidants and produced colorless 2,2-diphenyl-1-picrylhydrazine. The more colorless sample indicated the high antioxidant activity. DPPH and ascorbic acid were purchased from Sigma Chemical Co. Different concentrations of extracts were dissolved in ethanol and tested with freshly prepared 180 µl of DPPH in ethanol. The mixtures were then mixed with a vortex mixer and incubated in the dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 520 nm [7]. Ascorbic acid was used as standard.

The percentage of inhibition was calculated by the equation:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where, A_{control} is the absorbance of the control reaction and A_{sample} is the absorbance of the test sample. The half

maximal inhibitory concentration (IC_{50}) was calculated from the curve between the percentage of inhibition and the concentration of extract.

Determination of mushroom tyrosinase inhibition activity

Each extract was dissolved in ethanol at the concentration of 2.5, 100 µl of each sample was added to the 96-well plate and then 40 µl of 2.5 mM L-dopa solution were added to the well plate, then incubated at 37°C for 5 min before adding 60 µl of mushroom tyrosinase enzyme [1,8]. The mixture was incubated again at 37°C for 15 min before determining the absorbance at 450 nm with the microplate reader. Kojic acid was used as reference tyrosinase inhibitors. The percentage inhibition of tyrosinase activity was calculated as followed:

$$\text{Inhibition (\%)} = [(A_a - A_b) / A_a] \times 100$$

Where A_a = absorbance without a test sample and A_b = absorbance with a test sample.

Determination of TLC profile

Determination TLC profile was conducted using the TLC plate that has activated. Oxyresveratrol was used as standards. The extracts were performed for TLC fingerprints on Silica gel GF₂₅₄ plates. The eluent used combination of ethyl acetate and methanol (7:3). The chromatogram was detected at a wavelength of 254 nm and 366 nm [9].

Assay oxyresveratrol in extracts by HPLC

Oxyresveratrol were determined using HPLC (Shimadzu, Japan). All samples were solved in methanol and filtered through 0.45 µm filter paper. 10µL samples was injected into a C18 (*octadecisilan*) 150 x 4.6 mm, Mobile phase acetonitrile-formic acid 0.1% in water. Flow rate 1 mL/minute, UV Detector 327 nm, column temperature was maintained at 40°C. Chromatograms were recorded at 327 nm [10]. Identification of oxyresveratrol in extracts was performed by comparing the retention time and chromatogram with their reference standard compounds. Validation of analytical methods has been performed for the determination of oxyresveratrol levels.

Statistical analysis

The analyses were performed in triplicate, and the results expressed as mean \pm standard deviation (SD). Differences were considered significant when $p < 0.05$. Multiple comparisons

between more than two groups were performed with one-way ANOVA supplemented with Kruskal Wallis test. The data obtained were analyzed using the SPSS® version 18.0.

RESULTS AND DISCUSSION

Extraction

In this study, 85% ethanol extract of the roots of mulberry produce the highest yield. The order of solvent polarity from high to low polarity is E50, M60, E70, M85, M100, E96. Based on the results, the polarity of the solvent does not affect the resulting extract results, since there is no significant polarity difference.

Table 1 Crude Extract of Mulberry root

Solvent	Crude Extract (mg)	Yield (%)	Loss on drying (%)
E50	56.28	18.72	10.75
E70	47.15	15.65	10.28
E96	37.88	12.59	10.11
M60	58.15	19.31	14.70
M85	63.12	20.94	13.50
M100	46.85	15.54	12.41

Antityrosinase and Antioxidant Activity

The antioxidant activity of extracts of mulberry root was evaluated by DPPH assay, ascorbic acid was used as standard. DPPH assay is widely used for testing the ability of compounds that act as free radical scavengers or hydrogen donors. Mulberry root extract is used as active ingredients in anti-aging and whitening agent products due to their antioxidant activity. The results are shown in Table 2. A lower IC_{50} value revealed a good antioxidant activity. Ethanolic 96% extracts showed the higher antioxidant

activity was significantly different from other extracts.

Therefore, the research focus on the results of ethanolic 96% extracts. Ethanolic 96% mulberry root extracts showed higher activity than other extracts in the same species that may be related to the lower of yield extract, so have the higher active content. Therefore, the ethanolic extracts were selected for further study. Antityrosinase and antioxidant activity of 96% ethanol extract of mulberry root are stronger than the five other extracts. This result was similar to the results of previous studies which stated that the activity of DPPH scavenger with EtOH solvent showed a strong antioxidant activity that increased with increasing concentration of extract [12].

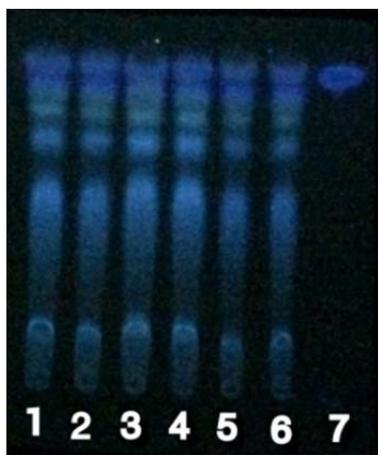
Tyrosinase enzyme plays an important role in melanin synthesis. It can change tyrosine to L-dopa, then convert to dopaquinone and with several polymerization reactions, eumelanin and pheomelanin are formed [8,11]. Compounds that can inhibit tyrosinase enzyme are used as skin brightening agent. The results are shown in Table 2. The ethanolic 96% extract revealed the highest activity ($IC_{50} = 75.37\%$). However, all the extracts presented a lower activity than kojic acid. These indicated that antioxidant compounds might promote the tyrosinase inhibition activity due to their antioxidative synergistic [8,11].

Table 2 IC_{50} Antityrosinase and Antioxidant Activity of crude extracts

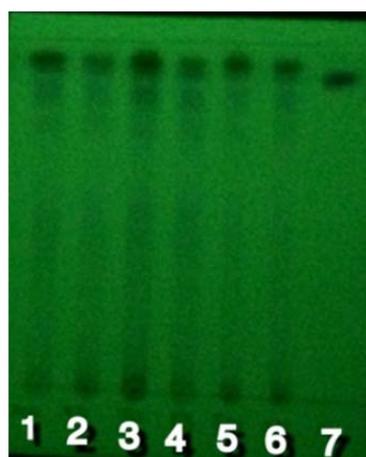
Solvent	IC_{50} ($\mu\text{g/ml}$) Antityrosinase activity	IC_{50} ($\mu\text{g/ml}$) Antioxidant activity
E50	119.84 \pm 1.99	86.41 \pm 2.30
E70	103.44 \pm 2.57	54.47 \pm 1.07
E96	75.37 \pm 1.91	50.34 \pm 0.43
M60	98.73 \pm 1.85	91.46 \pm 0.89
M85	86.03 \pm 1.17	69.47 \pm 0.20
M100	82.93 \pm 1.84	66.03 \pm 0.40
Ascorbic acid	-	6.78 \pm 0.05
Kojic acid	11.32 \pm 0.66	-

TLC Separation

In the TLC chromatograms shown in Figure 1 can be seen the same spot between the reference standard and sixth extract others at UV 254 nm and 366 nm, so that it can be concluded mulberry root containing oxyresveratrol.



(a)



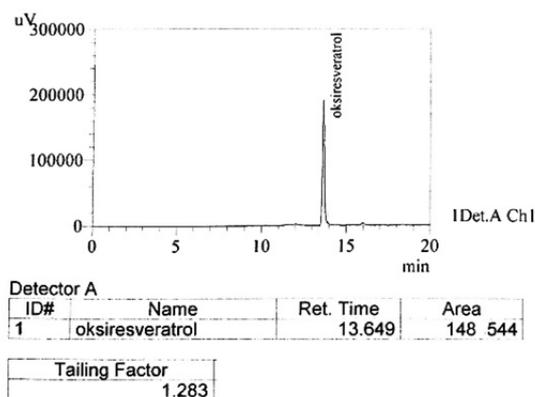
(b)

Figure 1. TLC profile of extract using UV 366 nm (a) and UV 254 nm (b). (1) E50 (2) E70 (3) E96 (4) M60 (5) M80 (6) M100 (7) Oxyresveratrol reference standard

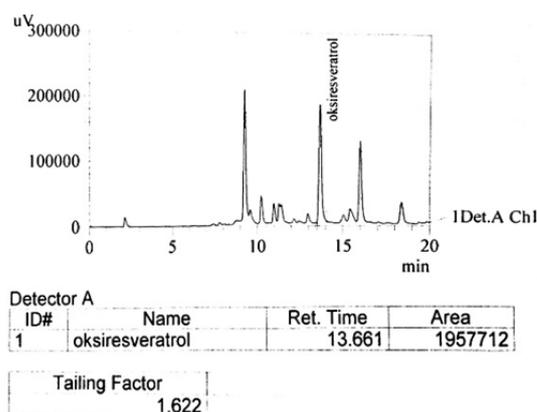
Assay of Oxyresveratrol in Extracts

HPLC system C18 150 x 4.6 mm, Mobile phase Acetonitril-formic acid 0.1% in water. Flow rate 1 mL/minute, UV Detector 327 nm, column temperature was maintained at 40°C. Eluent A (acetonitrile) and B (0.1% aqueous formic acid, v/v) were used with the gradient program set as follows: 0–20 min, linear change from A-B

(5 : 95 v/v) to A-B (30 : 70 v/v). Analysis oxyresveratrol using HPLC has done verification and gave accuracy with relative standard deviation of 0.76%, recovery rate 97.85%, and limit of quantitation 3.82 µg/ml, and linearity value was 0.9983.



(a)



(b)

Figure 2. HPLC Chromatograms of oxyresveratrol (a) and Mulberry root extract (b)

Table 3. Content of Oxyresveratrol in Crude Extract

Solvent	Oxyresveratrol (%)
E50	0.97 ± 0.010
E70	0.85 ± 0.006
E96	1.76 ± 0.081
M60	1.16 ± 0.043
M85	1.68 ± 0.005
M100	2.17 ± 0.005

Analyzing statistically the oxyresveratrol content using Kruskal Wallis method obtained a significant value of 0.006 with a confidence level of 95%. This indicates that there was a significant difference of oxyresveratrol content from mulberry root extract using different polarity solvent.

CONCLUSION

Antityrosinase and antioxidant activity of 96% ethanol extract of mulberry root are the most highest than other of the fifth extract. Oxyresveratrol level is the most highest in the methanol extract 100%. So, in this study, Antityrosinase and antioxidant activity of mulberry root extract was not related to oxyresveratrol content in extracts.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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