In-vitro antioxidant and antibacterial activities of tegeran wood (*Cudrania javanensis* Trécul) extracts

Aktivitas antioksidan dan antibakteri ekstrak kayu tegeran secara in-vitro (*Cudrania javanensis* Trécul)

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ABSTRAK

Cudrania javanensis pada umumnya digunakan sebagai zat warna alam di Indonesia, tetapi masih terbatas penelitian tentang aktivitas biologinya. Penelitian ini bertujuan untuk mempelajari tentang aktivitas antioksidan dan antibakteri ekstrak *C. javanensis*. Aktivitas antioksidan ekstrak diuji dengan metode DPPH dan ABTS. Aktivitas antibakteri ekstrak metanol dan air *C. javanensis* juga dievaluasi terhadap bakteri gram positif (*Staphylococcus aureus*) dan bakteri gram negatif (*Escherichia coli* dan *Pseudomonas aeruginosa*) dengan metode difusi agar. Hasil penelitian menunjukkan bahwa aktivitas antioksidan ekstrak metanol lebih tinggi dibandingkan dengan ekstrak air yang mampu menghambat radikal DPPH dengan IC_{50} sebesar 12,23±1,43 µg/mL dan penangkapan radikal ABTS sebesar 964,69±15,05 mg trolox equivalent/g pada konsentrasi 0,025 mg/mL. Akan tetapi, kedua ekstrak tidak dapat menghambat kedua jenis bakteri uji. Penelitian ini menunjukkan bahwa ekstrak metanol dan air *C. javanensis* berpotensi menjadi salah satu sumber antioksidan alami.

Kata Kunci: antioksidan; antibakteri; *Cudrania javanensis*; kayu tegeran

ABSTRACT

Cudrania javanensis generally used as natural dyes in Indonesia, but limited is known about its biological activities. The study aimed to assess in vitro antioxidant and antibacterial activities of C. javanensis crude extracts. The antioxidant properties of crude extracts were determined by the DPPH free radical and ABTS method. Methanol and water extracts were also evaluated for their antimicrobial activities toward strain of Gram positive bacteria (Staphylococcus aureus) and Gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa) by the agar well diffusion method. The results indicated that C. javanensis wood methanol extract exhibited good antioxidant activity than water extract, which against DPPH radical with IC_{50} of 12.23±1.43 µg/mL, and scavenging to ABTS radical about 964.69±15.05 mg trolox equivalent/g at 0.025 mg/mL, respectively. However, both of extracts did not possess activity toward antibacterial assay. This study indicated that methanol and water extracts from C. javanensis wood could be used as natural antioxidant resources.

Keywords: *antioxidant; antibacterial; C. javanensis; tegeran wood*

I. INTRODUCTION

Cellular metabolism and functional activities can produce reactive oxygen species (ROS). Increasing of ROS may term oxidative stress and significantly lead to many kinds of diseases such as cancer, cardiovascular, and degenerative diseases (Lü, Lin, Yao, & Chen, 2010; Halliwell, 2012). Antioxidant is a compound that can avoid or decrease the ROS-induced

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oxidative stress in the body. Plants produce some metabolites such as polyphenol, vitamin, polysaccharides, and terpenoid which are considered as antioxidant sources (Tusevski, Kostovska, Iloska, Trajkovska, & Simic, 2014). The most important metabolite which existed in seed, leaves, bark, root, and fruit is phenolic compounds (Nisa, Nurhayati, Apriyana, & Indrianingsih, 2017). In addition to as antioxidants, the phenolic compounds are also exhibited antibacterial activities (Islam et al., 2018; Park et al., 2019).

Cudrania javanensis Trécul is a member of Moraceae family. Other names of this plant are *Maclura cochinchinensis* (Lour.) Corner and *Cudrania cochinchinensis* (Sato, Chewchinda, Parichatikanond, & Vongsak, 2019). *M. cochinchinensis* extracts have been used for treatment of fever, skin infections, cough, gout, hyperuricemia, and inflammation (Kongkiatpaiboon et al., 2017; Sarmah & Sharma, 2010; Sato et al., 2019). Some research reported that this plant exhibited biological activities such as antibacterial, antioxidant, tyrosinase inhibitors, anti-inflammation, anti-cancer and antifungal (Chen et al., 2017; Chien et al., 2018; Darsih et al., 2019; Kummee & Intaraksa, 2008; Zheng, Zhu, Fan, Tan, & Wang, 2011). Several bioactive compounds on *C. javanensis* have been reported such as morin, prenylated isoflavones, resveratrol, and oxyresveratrol (Chen, Zhou, Li, Liu, & Dong, 2015; Chen et al., 2017; Chien et al., 2018).

In Indonesia, *C. javanensis* is used as natural dyes in fabrics, but there is only a little attention to its biological activities. The antifungal and phytochemicals of *C. javanensis* extracts have been reported by Darsih et al. (2019), however, the antioxidant properties and antimicrobial against bacteria of the extracts have not yet been studied. The aim of this study was to assess in vitro antioxidant properties and antibacterial of *C. javanensis* crude extracts.

II. MATERIALS AND METHODS

2.1. Materials

Cudrania javanensis (tegeran wood, local name) was bought from the market in Ngasem, Yogyakarta, Indonesia.

The chemicals and biological materials were used in this study are Potato Dextrose Agar (PDA), Dimethyl Sulfoxide (DMSO), methanol, distilled water, 2,2-diphenyl 1 picrylhidrazyl (DPPH), ascorbic acid, diammonium 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate (ABTS), potasium persulfate, and trolox.

The bacterial were used in this research are strain of gram-positive bacteria (*Staphylococcus aureus* FNCC 0047) and gram-negative bacteria (*Escherichia coli* FNCC 194 and *Pseudomonas aeruginosa FNCC 0063*).

2.2. Methods

2.2.1 Preparation of *C. javanensis* crude extracts

The *C. javanensis* wood were chopped into small size and then macerated with methanol solvent for 24 hours, with no repetition, with ratio 1:10 w/v. Another extract was obtained by boiling the wood with distilled water at temperature 100°C for 1 hour (with ratio 1:10 w/v). The extraction of *C. javanensis* wood was carried out in the previous article (Darsih et al., 2019).

2.2.2 Antioxidant activity (DPPH method)

The antioxidant activity of *C. javanensis* extracts was determined using DPPH method. The 20 µL of extracts placed in 96-well plate were treated with 180 µL DPPH solution (40 mM in methanol) and incubated for 30 minutes in the darkness at temperature 25°C. Then the absorbance of solutions was measured with ELISA reader at 517 nm. Ascorbic acid was used as a positive control. The equation to calculate the ability of the extracts to scavenge the DPPH radicals can be seen in equation (1).

$$\%inhibition = \frac{(A0-A1)}{A0} x100 \ldots\ldots\ldots (1)$$

Where the control was expressed as A0, and the presence of the samples on the solution was revealed as A1 (Indrianingsih, Tachibana, & Itoh, 2015). The IC_{50} was calculated using curve of % inhibition DPPH at various concentrations, following the formula y= ax + b, where y=50 and x is the value of IC_{50}.

2.2.3 ABTS method

Trolox equivalents were assessed using the ABTS method (Gan, Xu, Song, Kuang, & Li, 2010; Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006) with slight modification. Briefly, 7.4 mM diammonium 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS) and 2.6 mM potasium persulfate (1:1 v/v) were used to prepare the ABTS•+ stock solution. Then the stock solution was kept in the darkness at room temperature for 16 hours. The absorbance of ABTS solution at 734 nm (1.1±0.02 units) was obtained by mixing 1 mL ABTS•+ stock solution with 60 mL methanol and equilibrated at 30°C. In 96-well plate, 15 µL samples were received with ABTS•+ solution in total volume 300 µL. The concentration of the samples used in this research were 0.025; 0.075; and 0.1 mg/mL. After 2 hours of incubation in the darkness, the samples and standard absorbance were measured using ELISA reader at 734 nm. Trolox was used as standard. The equation to calculate the ability of the extracts to scavenge the ABTS radicals can be seen in equation (2).

$$\%inhibition = \frac{(A0-A1)}{A0} x100 \ldots\ldots\ldots (2)$$

Where A0 showed the absorbance of control, and A1 showed the absorbance of samples/standard. The standar curve was linear between 0.05 and 0.175 mg/mL Trolox. Results are expressed in mg Trolox equivalent (TE)/ g.

2.2.4 Antibacterial activity

Antibacterial activity of *C. javanensis* extracts toward gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*) was determined by agar well diffusion method (Balouiri, Sadiki, & Ibnsouda, 2016). Inoculum of test microbial (100 µL) was spread on the surface of agar plate. Then 25 µL of extracts with various concentrations (500, 750, 1000 µg/mL) were added in the hole of the agar plate. The inhibition zones of extracts were measured after 24 hours incubation at incubator with the temperature of 37°C.

III. RESULTS AND DISCUSSION

Table 1 shows the scavenging activity of *C. javanensis* crude extracts toward DPPH radicals. Both extracts showed strong antioxidant activity with IC_{50}< 50µg/mL (Zhu, Lian, Guo, Peng, & Zhou, 2011). The methanolic extract exhibited a higher antioxidant activity compared to water extract, but lower than ascorbic acid as control (IC_{50} 8.28±0.64 µg/mL). The ascorbic acid as control exhibited a strong antioxidant activity. The antioxidant activity of methanol extract was higher than the water because total polyphenol on this extract was higher than the water extract (Table 1). Morin as a major compound on *C. javanensis* was assumed responsible for antioxidant activity (Leakaya et al., 2018). The antioxidant activity of *C. javanensis* extracts against ABTS radical also exhibited good activity (Figure 1). At the highest concentration of 0.1 mg/mL, the methanolic extract showed the ABTS scavenging activity as 2148.056 mg Trolox equivalent/g, while the water extract presented 1956.576 mg Trolox equivalent/g. The results showed that the scavenging activity increased with the concentrations of extracts. The phenolic compounds on *C. javanensis* extracts was suggested to contribute to antioxidant properties. The phenolic compounds have ability to donate hydrogen, quench singlet oxygen and act as metal chelators, which can act as antioxidant agents (Nisa, Nurhayati, Apriyana, & Indrianingsih, 2017). This result was similar to those reported in previous research, the scavenging to DPPH and ABTS radicals correlates directly with the polyphenols content (Leakaya, Sato, & Chewchinda, 2018; Sato et al., 2019).

Table 1. Scavenging activity toward DPPH radical of *C. javanensis* extracts

Sample	IC_{50} (µg/mL)	Total polyphenol (mg GAE/g) [a]
Methanol	12.23±1.43	484.72
Water	14.68±0.20	389.90
Ascorbic acid	8.28±0.64	NA

[a]Darsih et al. (2019)
The IC_{50} values are shown as the mean±SD (n=3)

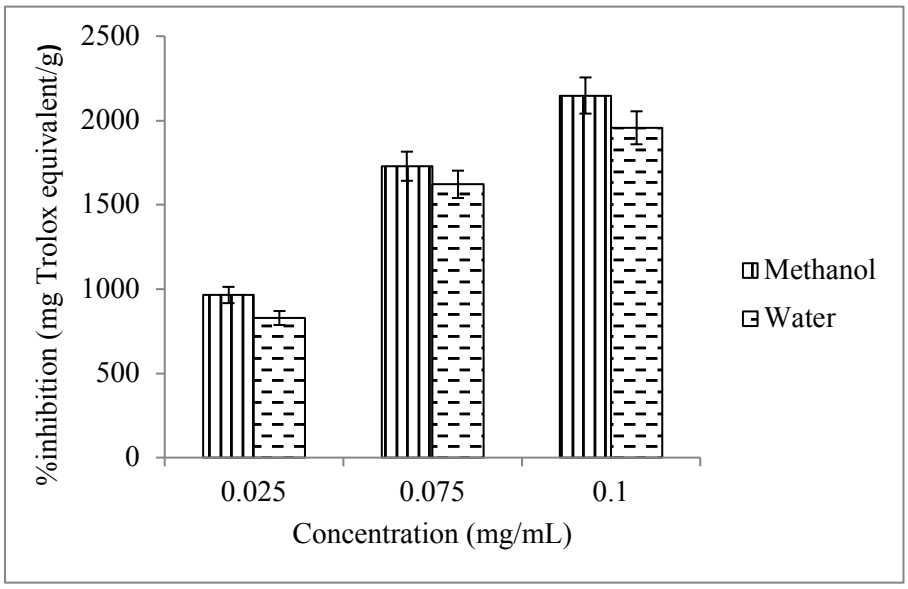

Figure 1. The comparison of ABTS radical scavenging of *C. javanensis* extract. The inhibition values are shown as the mean±SD (n=3)

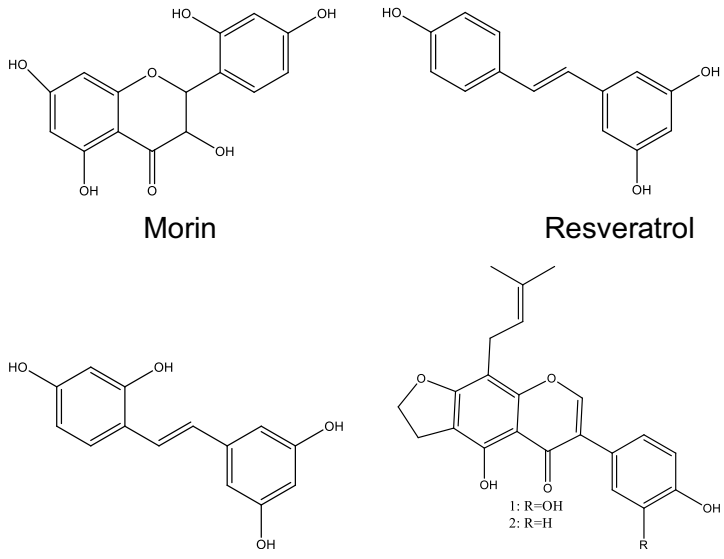

Figure 2. Active compounds that have been isolated from *C. javanensis* (Chen, Zhou, Li, Liu, & Dong, 2015; Y. Chen et al., 2017; Chien et al., 2018).

Table 2. Inhibition zone of *C. javanensis* extracts toward *S. aureus*, *E. coli* and *P. aeruginosa*

Samples	Concentration (µg/mL)	Inhibition zone (mm)		
		S. aureus	*E. coli*	*P. aeruginosa*
Methanol	500	-	-	-
	750	-	-	-
	1000	-	-	-
Water	500	-	-	-
	750	-	-	-
	1000	-	-	-
[a]Chloramphenicol	100	9.63±0.15	-	11.68±0.58
	250	12.80±0.12	13.17±0.50	12.75±0.26
	500	15.48±0.67	16.60±0.12	17.98±0.25

[a]Chloramphenicol : positive control
(-) : no inhibition

Bioactive compounds on *C. javanensis* can be seen ini Figure 2. These components may have the potential for antioxidant activity in this study. A literature also reported that morin as the major compound on *C. javanensis* was assumed responsible for antioxidant activity (Leakaya et al., 2018). The active compounds in *C. javanensis* have not been widely studied, however, there was also a literature that isolated active compounds from another *Cudrania* species, namely *Cudrania fruticosa* Wight. The results of investigations with various chromatographic, NMR and mass spectrometry revealed that roots of *C. fruticose* contain xanthones (Liang et al, 2015). Another research on Cudrania species, namely *Cudrania tricuspidata*, also succeeded in isolating an isoflavonoid compound, namely 6,8-diprenylgenistein (DPG) from the fruits. This compound was indicated to have an antidiabetic activity that has been tested on mice (Jo et al, 2015). The antibacterial activity of *C. javanensis* extracts were shown in Table 2. *C. javanensis* extracts did not exhibit antimicrobial against gram-negative bacteria at 500, 750, and 1.000 µg/mL. This is suggested that extracts contained metabolites which are not active against the test bacterial. This result was similar to the research about antibacterial activity of *Maclura cochinchinensis* extracts toward *S. cusia* against *E. coli*, *P. aeruginosa*, *Salmonella typhi*, and *Shigella sonnei* with concentration extract of 2 mg/disc. Both extracts were also not active toward *S. aureus*. This result was opposite to the previous research about antibacterial activity of *M. cochinchinensis*. Previous research reported that the methanol and water extracts showed activity against *S.aureus* with inhibition zone 12.8 mm and 8.5 mm, respectively (Kummee & Intaraksa, 2008). This is suggested the different concentration of metabolites on *C. javanensis* extracts. The various metabolites of plant and its concentration were affected by environmental, season, and geographical location. Previous research reported that total phenolic content (TPC) from aqueous *C. javanensis* heartwood extract from Thailand with value of 1.9 g GAE/ g (Leakaya et.al., 2018). This value was higher than TPC of *C. javanensis* extracts from Indonesia.

IV. CONCLUSIONS

Methanolic and water extracts of *C. javanensis* showed strong antioxidant properties, otherwise, both extracts were not active against microbial. The antioxidant properties of extracts were suggested related to the total polyphenols content. These extracts possessed significant antioxidant activity which is prospected to be antioxidant resources.

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