Content

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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF LEAVES EXTRACTS OF CORDYLINE FRUTICOSA BACK

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Abstract: *Cordyline fruticosa* Back is traditionally used for treatment of various diseases in Indonesia. The leaves of *C. fruticosa* Back were collected from East Kalimantan Province, Indonesia, and extracted by different polarity solvents: methanol (MeOH), ethyl acetate (EtOAc) and hexane to determine their antioxidant and antibacterial activities. The DPPH radical scavenging assay identified that all extracts had potent antioxidant activity (IC_{50} 0.94-8.19 mg/mL) with the EtOAc extract showing the highest antioxidant activity. All extracts also presented the potent antioxidant activity in the Fe^{2+} chelating assay (IC_{50} 0.91-3.86 mg/mL) with the highest activity found in hexane extract. In the FRAP assay, the EtOAc extract exhibited the strongest ferric reducing power activity (31.36 ± 0.21 mmol Fe^{2+} equivalents (eq)/1 g sample) followed by MeOH extract (15.83 ± 1.96 mmol Fe^{2+} eq/g) and hexane extract (9.58 ± 0.38 mmol Fe^{2+} eq/g). In the Folin-Ciocalteau assay, the MeOH extract showed the highest total phenolic content value (41.91 ± 1.32 mg gallic acid equivalent (GAE)/1 g sample) followed by hexane extract (24.62 ± 0.84 mg GAE/g) and EtOAc extract (23.16 ± 0.94 mg GAE/g). In addition, the MeOH extract displayed antibacterial activity against *Bacillus cereus, Salmonella thypii* and *Streptococcus sobrinus* with a diameter of inhibition zone of 9.1, 8.1 and 8.1 mm, respectively at a concentration of 500 ppm. These results indicate that *C. fruticosa* Back is potentially used for natural antioxidant and antibacterial agents.

1. Introduction

The genus *Cordyline*, with about 20 species, is widely distributed in Southeast Asia, Australia and New Zealand [1]. Cordylines are known to the world by many names and extensively used in Southeast Asia for the treatment of various diseases [2]. In Indonesia, *Cordyline fruticosa* Back (Liliaceae), locally called "Andong", has been used in folklore medicine to treat various diseases utilizing the roots and leaves [3,4]. Some scientific data reported that Cordylines had a potential activity as antioxidant [4], cytotoxic agent [5,6], antimicrobial [6,7] and anthelmintic [8]. However, there are no reports describing the biological activity from *C. fruticosa* Back, specifically.

Preliminary screening of ethanol extract of *C. fruticosa* (L.) A. Cheval showed that it had scavenging activity against DPPH about 283.82 ± 1.02 μg/mL [4]. The isolated saponin from MeOH extract were not able to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, nevertheless it showed a moderate antibacterial activity against the Gram-positive *Enterococcus faecalis* [6].

Due to the fact that *C. fruticosa* Back has been used as a traditional medicine and there are not any reports on its biological activities, the antioxidant and antibacterial activities are investigated and reported herein.

2. Materials and Methods

2.1 Instruments and Chemicals

UV spectra were recorded on a PerkinElmer Instruments Lamda 35 UV/Vis Spectrometer. 1,1-Dipheny-2-picrylhydrazyl (DPPH), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4"-disulfonic acid mono-sodium salt (ferrozine), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and Folin-Ciocalteau reagent were purchased from Fluka or Sigma-Aldrich.

2.2 Plant Sample

The leaves of *C. fruticosa* Back were collected from East Kalimantan Province, Indonesia, in June 2013. The specimen was identified by Technology Research Institute of Natural Resources Conservation, Indonesian Forestry Ministry, and the voucher specimen was deposited at Laboratory of Forest Product Chemistry, Faculty of Forestry, Mulawarman University, Indonesia, with voucher number CF-DD-3.

2.3 Extract Preparations

The dry leaves (432 g) were extracted with 6 L MeOH to afford the MeOH extract. The MeOH extract (49.2 g) was successively extracted three times with 0.7 L of ethyl acetate (EtOAc) and hexane. The mixture was filtered and the combined filtrate was concentrated under reduced pressure to yield the EtOAc and hexane extracts, respectively.

2.4 DPPH Radical Scavenging Activity

The scavenging activity of extracts for the DPPH radical was measured as described [9] with some

modifications. Each *C. fruticosa* Back extract was dissolved in MeOH at a concentration of 0.01-16.0 mg/mL. Various concentrations of extracts (100 μL) were mixed with 3 mL of DPPH methanolic solution (60 μM). The solution was stirred and left to stand for 20 minutes in the dark and measured the absorption at 517 nm. The percentage inhibition was calculated using the equation: % Inhibition = $[(A_1-A_2)/A_1] \times 100$, where A_1 and A_2 are the absorbance of DPPH and extract solution, respectively. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentrations. Ascorbic acid was used as a standard. All experiments were performed in triplicate.

2.5 Ferrous (Fe^{2+}) Chelating Activity Assay

The chelating activity on ferrous ion was adapted according to the method [10] with a minor modification. The reaction contained 1 mL of sample at various concentrations (0.01-6.5 mg/mL) with 1 mL of 0.1 mM $FeSO_4$ mixed for 30 seconds, then 1 mL of 0.25 mM ferrozine was added and the mixture was incubated for 10 minutes at room temperature. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. EDTA-2Na was used as the standard. The chelating activity of the extract was calculated as: % Chelating rate = $[1-(A_1-A_2)/A_0] \times 100$, where A_0 is the absorbance of control (without extract), A_1 is the absorbance of sample with ferrozine and A_2 is the absorbance of sample without ferrozine.

2.6 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing power was determined using a modified version of the FRAP assay [11]. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6) with 10 mM TPTZ in 40 mM hydrochloric acid and 20 mM ferric chloride (10:1:1). The freshly prepared FRAP reagent (1.5 mL) was added to 50 μL of sample along with 150 μL distilled water. The reaction mixture was incubated for 30 minutes in room temperature. Then, the absorbance of the sample was measured at 593 nm. A standard curve ($y = 0.012x + 0.448$; $r^2 = 0.985$) was prepared using various concentrations of $FeSO_4.7H_2O$ (5-30 mM). All measurements were done in triplicate. FRAP values were expressed as mmol Fe^{2+} equivalents (eq)/1 g sample.

2.7 Total Phenolic Content (TPC)

The total phenol content was obtained by a modification of the Folin-Ciocalteau method [12]. A 200 μL of sample was dissolved in MeOH : H_2O (1:1), mixed with 1 mL of 0.2 N Folin-Ciocalteau reagent and stirred for 5 minutes. Then, 80 μL of 7.5 % sodium carbonate (Na_2CO_3) was added and the mixture was incubated at room temperature in the dark for 1 hour. The absorbance was measured at 760 nm. Gallic acid was used as a calibration curve ($y = -10^{-5}x^2 + 0.007x - 0.017$; $r^2 = 0.998$) by various concentrations (20-220 μg/mL). All experiments were performed in triplicate. The results are expressed as mg gallic acid equivalents (GAE)/ 1 g sample.

2.8 Antibacterial Activity

Antibacterial activity was conducted using the disc diffusion method as previously described [13] with minor modification. Nutrient dextrose agar was used as a media. MeOH extract (500 ppm) was used to investigate the antibacterial activity against *Bacillus cereus*, *Salmonella thypii* and *Streptococcus sobrinus*. The plates were kept in an incubator at 37°C for 24 hours. Chloramphenicol was used as a standard drug to ensure the activity of standard antibiotic against the test organisms. The diameters of zones of inhibition were measured in mm.

3. Results and Discussion

3.1 Extract Preparations

The dried leaves of *C. fruticosa* Back collected from East Kalimantan Province, Indonesia, were pulverized and macerated in MeOH for 72 hours. The filtrate was concentrated under reduced pressure to yield the MeOH extract. The MeOH extract (49.2 g) was partitioned with EtOAc and hexane to give EtOAc (1.8 g) and hexane extracts (9.5 g), respectively. Then, the crude MeOH and other partitions extracts were examined for total phenolic content and antioxidant activity. The results showed that the leaves of *C. fruticosa* Back may contain lots of high polarity compounds in the MeOH extract such as polyphenols, saponins, terpenoids and tannins [14].

Table 1: Antioxidant Activity and Total Phenolic Content of *Cordyline fruticosa* Back Extracts

Sample Extracts	**IC_{50} (mg/mL)**		**FRAP (mmol Fe^{2+} eq/g sample)**	**TPC (mg GAE/g sample)**
	DPPH Radical Scavenging	**Fe^{2+} Chelating Activity**		
MeOH	3.65 ± 0.04	3.86 ± 0.04	15.83 ± 1.96	41.91 ± 1.32
EtOAc	0.94 ± 0.01	2.44 ± 0.06	31.36 ± 0.21	23.16 ± 0.94
Hexane	8.19 ± 0.34	0.91 ± 0.01	9.58 ± 0.38	24.62 ± 0.84
Ascorbic Acid[a]	0.06 ± 0.00	-	-	-
EDTA-2Na[b]	-	0.03 ± 0.00	-	-

[a] Standard of DPPH scavenging assay; [b] Standard of Fe^{2+} chelating activity.

3.2 Antioxidant Activity

The results of DPPH radical scavenging, Fe^{2+} chelating and reducing power activity of *C. fruticosa* Back extracts are shown in Table 1. All extracts presented the potent antioxidant activity in the DPPH radical scavenging assay (IC_{50} 0.94-8.19 mg/mL) with the highest activity found in EtOAc extract. The Fe^{2+} chelating assay also identified that all extracts had potent antioxidant activity (IC_{50} 0.91-3.86 mg/mL) with the hexane extract showing the highest antioxidant activity. In the FRAP assay, the EtOAc extract exhibited the strongest ferric reducing power activity (31.36 ± 0.21 mmol Fe^{2+} equivalents (eq)/1 g sample) followed by MeOH extract (15.83 ± 1.96 mmol Fe^{2+} eq/g) and hexane extract (9.58 ± 0.38 mmol Fe^{2+} eq/g). These results indicate that the active compounds which have contribution on antioxidant activity in *C. fruticosa* Back may come from non-polar compounds.

3.3 Total Phenolic Content

In the Folin-Ciocalteau assay, the MeOH extract showed the highest total phenolic content value (41.91 ± 1.32 mg gallic acid equivalent (GAE)/1 g sample) followed by hexane extract (24.62 ± 0.84 mg GAE/g) and EtOAc extract (23.16 ± 0.94 mg GAE/g). Otherwise, the MeOH extract did not present the strongest antioxidant activity than others. This finding indicates that the phenolic compounds may not take a role as antioxidant in *C. fruticosa* Back, however the chlorophyll compound might contribute on the antioxidant activity rather than phenolic compounds [15].

3.4 Antibacterial Activity

The MeOH extract of *C. fruticosa* Back leaves were screened for its antibacterial activity against *Bacillus cereus*, *Salmonella thypii* and *Streptococcus sobrinus* at a concentration of 500 ppm (Table 2).

Tabel 2: The Antibacterial Activity of *Cordyline fruticosa* Back Extract

Sample Extracts	Inhibition Zone (mm)		
	B. cereus	***S. thypii***	***S. sobrinus***
MeOH	9.1 ± 1.3	8.1 ± 0.2	8.1 ± 0.2
Chloramphenicol[a]	18.6 ± 0.3	20.0 ± 0.6	18.1 ± 0.2

[a] Standard drug

The MeOH extract showed low activity against *B. cereus*, *S. thypii* and *S. sobrinus* with a diameter of inhibition zone of 9.1, 8.1 and 8.1 mm, respectively. This result reveals that *C. fruticosa* Back displays low activity against these bacterial strains.

4. Conclusions

The extracts of the leaves of *C. fruticosa* Back, collected from East Kalimantan Province, Indonesia, exhibited the potent antioxidant activity, but were low antibacterial activity.

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