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Antioxidant Activity of Daemonorops draco Resin

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Abstract

Jernang resin is secretion of jernang rattan (*Daemonorops draco*, *Arecaceae* family) fruits which is endemic in Southeast Asia. This resin has various biological activities and empirically used as wound healing, headache medicines, and fever remedies by Anak Dalam ethnic group from Jambi. This study was performed to evaluate the antioxidant activity of nonpolar fraction of *D. draco* resin which collected from Jambi Province, Sumatera, Indonesia. Resin was extracted with n-hexane, ethyl acetate, and methanol respectively. The antioxidant properties of the extracts were then evaluated using 1,1-diphenyl-2picryl-hidrazyl radical scavenging assay. The most active extract was further fractionated using n-hexane and methanol and separated using column chromatography and preparative thin layer chromatography. Separation of the extract was conducted through antioxidant assay-guided fractionation. Characterization of the active fraction was carried out by infrared spectroscopy. The result shows that ethyl acetate extract provides higher antioxidant activity (IC_{50} = 27.61 µg/mL) compare to methanol and n-hexane extracts. N-hexane fraction of ethyl acetate extract used for further separation using column and preparative thin layer chromatography due to its antioxidant activity. Separation using column chromatography resulting in 9 fractions (F.1-9). Fraction F.5 provide high antioxidant activity (IC_{50} = 17.27 µg/mL) and further separated using preparative thin layer chromatography resulting two fractions with lower antioxidant activity F.5.1 (IC_{50} = 85.18 µg/mL) and F.5.2 (IC_{50} = 34.94 µg/mL). Characterization of fraction F.5.2 using infrared spectroscopy showed that component in fraction F.5.2 contains NH-substituted benzene.

1. Introduction

Most of world's health problems are caused by the presence of free radicals. Uncontrolled free radicals development causes damages in tissues and biomolecules extensively, which lead to pathological disorders, such as aging [1], cancer [2], cardiovascular [3] and alzheimer disease [4]. Therefore, natural antioxidant is required as a free radicals scavenger and able to prevent the human body from any kind of oxidative damages. Natural resins from natural sources, which can be described as a sticky yet water-insoluble materials produced from damaged or infected plants, are acknowledged have a potential antimicrobial, antibacterial antibiofilm [5] and antioxidant activities [6, 7].

One of natural resin was produced in *Daemonorops draco* plant from Indonesia and well known as "Dragon's blood" resin. It was a bright red resin, which has been continuously used as dyes, varnish, incense and medicine [8]. Dragon's blood resin generally obtained from two main genus: *Daemonorops* (Palmae) found in Indonesia, India and Malaysia, and *Dracaena* (Liliaceae) grown in Hainan province of China, Vietnam, and Cambodia. Indonesian *D. draco* is considered as the most common species available for traditional medication, for instance, it was used for healing a wound, headache, and even fever by Anak Dalam ethnic group from Jambi, Indonesia [9]. Furthermore, *D. draco is* commercially produced by Meer corporation has been reported for having antibacterial activity [10]. *D. draco* from Jambi Province, Indonesia is also reported has antibacterial activity [11]. Moreover, it has been reported that *D. draco* resin also has antiviral [8], anticancer [12], and anti-inflammatory activity [13]. Activity of the resin as antioxidant need to be explored. This study was conducted to evaluate antioxidant activity of *D. draco* resin collected from Jambi Province, Sumatera,

Indonesia. Since some polar constituent from *D. draco* had been isolated [10, 14-16], this study also needs to evaluate nonpolar constituent of *D. draco* resin.

2. Methods

2.1. Extraction and Fractionation

D. draco resin was collected from Sarolangun Jambi, Sumatera, Indonesia. Approximately 150 g dried resin was extracted by 300 mL of solvents with a gradual increase of polarity in each solvent. Firstly, *n*-hexane was used, then ethyl acetate, and finally methanol as polar solvent. Ethyl acetate extract (1 g) was then fractionated by solvent-solvent extraction (methanol:*n*-hexane = 30 mL:30 mL) for 4 times, then the *n*-hexane fraction was collected. A total of 1.45 gram of *n*-hexane fraction was then separated by column chromatography with silica gel as the stationary phase and chloroform:*n*-hexane (9:1) as the mobile phase. From this separation step, 9 fraction was collected (number 1-9). Fraction 5 was again separated by preparative thin layer chromatography using GF_{254} silica glass plate and dichloromethane:*n*-hexane:methanol (9:1:0.1) solvent system, which resulting fraction 5.1 and fraction 5.2.

2.2. Antioxidant Assay

Antioxidant activity was carried out by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay. Each 100 µL of sample and DPPH solution (11.8 mg in 100 mL ethanol) were added into 96-well of microplate. The absorbance was then measured at 514 nm after 30 minutes of incubation. Finally, the inhibition activity of sample was calculated and determined [17]. In this assay, ascorbic acid was used as a positive control and ethanol was used as blank.

2.3. TLC Bioautography

Briefly, 10 µL of each extract (2 g in ethanol) was applied to the TLC Silica GF_{254}. Chromatography method was conducted using chloroform: methanol: water =9:1:0.1 as mobile phase. The TLC plate was then sprayed by DPPH solution following chromatographic elution. After 30 min, the yellow spots from reduced DPPH were clearly observed against the purple background.

2.4. Characteritation of Fraction

Characterization was conducted by infrared spectroscopy. 2 mg of sample was measured using attenuated total reflectance (ATR) FTIR spectrophotometer.

2.5. Statistical Analysis

Statistical analysis was performed as means ± SD from three independent replicates. One ways analysis of variance (ANOVA) was applied for comparison of the mean values with 95% confidence levels.

3. Results and Discussion

3.1. Extract and Fraction of *Daemonorops draco*

The extracts yields of *D. draco* resin using *n*-hexane, ethyl acetate, and methanol as extraction solvent were 0.22%, 73% and 6.38%, respectively (Table 1). The previous study reported by Hao *et.al.* [14] showed that the extraction of *D. draco* using chloroform give 87.33% yield. The result indicated that *D. draco* resin contains mainly semipolar constituents followed by the polar and then the nonpolar constituent. Ethyl acetate is a semipolar solvent with the index polarity of 4.4, likewise the chloroform. Those semipolar solvent successfully extracted constituents from *D. draco* resin.

Some of the semipolar and polar constituent from *D. draco* resin had been isolated and some of them are known to be the flavonoid compounds. Dracorhodin and dracorubin were isolated from chloroform-methanol extract [10], dracoflavan B1, B2, C1, C2, D1 and D2 were isolated from ethyl acetate extract [15], daemonorol group (A-F) were isolated from acetone extract [16] and dimethoxyflavan group were isolated from chloroform extract [14]. Since the compounds were mainly isolated from semipolar and polar extracts, it was predicted that the constituents in ethyl acetate and methanol extract should provide antioxidant activity.

Flavonoids play an important role in plant growth and development, and as the defense system of plants against the microorganisms and pests. The best-described property of almost every group of flavonoids is their ability to act as antioxidants. The flavonoids seem to be the most powerful compound to protect the body against reactive oxygen species [18].

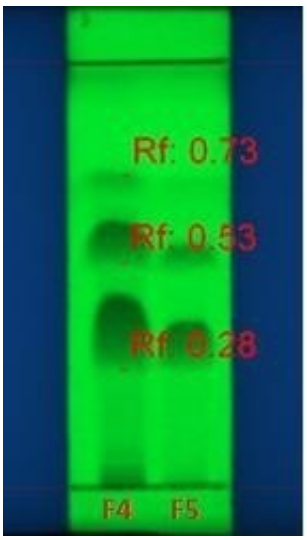

Figure 1. TLC chromatogram of fraction 4 and 5 at UV 254 nm

The ethyl acetate extract provided the highest maceration yield and was fractionated by solvent-solvent extraction with methanol and *n*-hexane. This separation yielded 24.46% of *n*-hexane fraction. The nonpolar fraction was further fractionated by column chromatography with the eluent system of chloroform:*n*-hexane (9:1) and obtained 9 fractions (1-9), where the fraction number 6 was provided the greatest yield. Furthermore, fraction number 4 showed better antioxidant activity followed by fraction number 5, but the difference was not significant ($p>0.05$). TLC profile (Figure 1) showed that fraction 4 has more components than those in fraction number 5. In order to obtain greater

weight and higher purity of active component, fraction number 5 was then separated with the preparative TLC using dichloromethane: *n*-hexane: methanol (9:1:0.1) eluent system.

Table 1. Weight, % yield, and antioxidant activity of extracts and fraction of D. draco

Sample	Weight (g)	% Yield	IC_{50} (µg/mL)
n-hexane extract	0.2950	0.22	63.06±2.28
Ethyl acetate extract	100.2595	73.31	27.61±0.40
Methanol extract	8.7293	6.38	63.86±5.40
Non polar fraction	1.4510	24.46	-
F.1*	0.0105	0.72	5697.49±500.94
F.2*	0.0033	0.23	651.86±33.21
F.3*	0.0905	6.24	56.93±9.55
F.4*	0.1124	7.75	14.19±2.04
F.5*	0.1122	7.73	17.27±4.31
F.6*	0.1302	8.97	78.34±7.91
F.7*	0.0289	1.99	94.20±18.81
F.8*	0.0265	1.83	21.20±1.15
F.9*	0.0179	1.23	81.41±8.08
F.5.1**	0.0036	9.00	85.18±0.52
F.5.2**	0.0057	14.25	34.94±2.88
Ascorbic acid			5.12±0.15

* Separated by column chromatography
**Separated by preparative TLC
(-) not tested

3.2. Antioxidant Activity of Extracts and Fractions of *Daemonorops draco*

The antioxidant activities of *D. draco* extracts were evaluated by their ability as the DPPH radicals scavenging through the hydrogen donating mechanism, which could reduce the stable violet of DPPH radical to the yellow DPPH-H. The result in Table 1 showed that the ethyl acetate extract of *D. draco* exhibited higher antioxidant activity (IC_{50} 27.61 µg/mL) than the activity of *n*-hexane and methanol extract.

The antioxidant activities of *D. draco* showed at IC_{50} values range of 14.19-569.49 µg/mL (Table 1). Other species showed IC_{50} such *Dracaena cinnabari* Balf f. resin (IC_{50} 94.2-135.7 µg/mL) [7], *Pinus oocarpa* (154.50 µg/mL), *Pinus insularis* (99.328 µg/mL), *Pinus merkusii* (60.20 µg/mL) and *Agathis loranthifolia* (245.99-438.55 µg/mL) [6], it showed that *D. draco* extracts has a higher activity. However, it still has a lower activity than ascorbic acid (IC_{50} 5.12±0.15 µg/mL).

A qualitative test of the extracts was performed to identify the compounds which contribute in the antioxidant activity by using thin layer chromatography method followed by bioautography method using DPPH reagent, where active compound was identified as a pale-yellow spot on a violet background. Component with the highest DPPH reducing activity shown in Figure 2C, was a component with the retardation factor (R_f) of 0.71 (indicated with red arrow) in *n*-hexane, ethyl acetate, and methanol extract, which was then become the targetted component in this research. Fractionation was then conducted by solvent-solvent extraction method (methanol:*n*-hexane) in order to increase the component yield. The *n*-hexane fraction was then further fractionated by column chromatography.

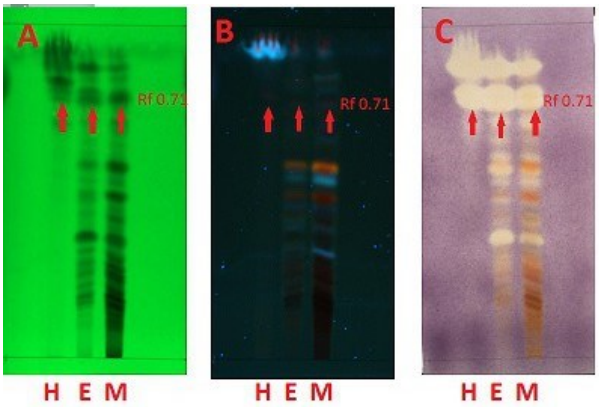

Figure 2. TLC chromatogram of D. draco resin extract monitored at 254 nm (A), 366 nm (B), visible light after sprayed by DPPH, antioxidant bioautography (C), n-hexane extract (H), ethyl acetate extract (E), methanol extract (M), the mobile phase was chloroform: methanol: water = 9:1:0.1, while the stationary phase was silica GF_{254}

TLC-bioautography showed that the component with R_f of 0.71 posses antioxidant activity. Solvent-solvent extraction was conducted to separated the nonpolar from the polar component. The nonpolar component was then fractionated by column chromatography. According to Table 1, Fraction 4 from column chromatography showed the highest antioxidant activity but not significantly different with Fraction 5 ($p>0.05$). From the TLC profile, it showed that there are more components in Fraction 4 than the components in the Fraction 5, therefore in order to obtain the active component with a greater yield, the separation of Fraction 5 with less components was carried out by preparative TLC. Fraction 5 (IC_{50}: 17.27 ± 4.31 µg/mL) has a better antioxidant activity than F.5.1 and F.5.2. It is estimated that there is a synergistic effect between the compounds in fraction number 5 in their mechanism as an antioxidant.

Fraction 5.2, which has an active component as antioxidant, has R_f 0.30 in TLC with F_{254} silica gel as the stationary phase and dichloromethane:n-hexane:methanol (9:1:0.1) as the mobile phase. Fraction 5.2 is a brownish-white amorphous solid which has a melting point at 160-162 °C. The results of the analysis of F.5.2 with FTIR spectrophotometer (Figure 3) shows the absorption at wave number of 3445 cm^{-1} as one single peak which suggested that there is N-H band of the secondary amine. The absorption at 1600 cm^{-1} indicates the presence of aromatic groups. The absorption that appears at 2843 cm^{-1} shows the vibration of the C-H strain. Vibration of C=C ring indicated at 1500 cm^{-1} and 1458 cm^{-1}. This result indicates that F.5.2 contains amine ($-NH_2$) substituted aromatic group.

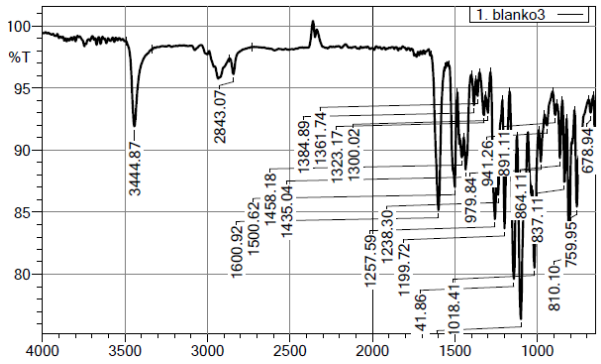

Figure 3. IR spectrum of F.5.2

The UV spectrum of F.5.2 showed the peak in the wavenumber of 230 nm indicating a transition of π→π* and the peak at 280 nm indicating a forbidden transition of π→π*. Therefore, it roughly indicates that F.5.2 contains NH-substituted benzene. A further isolation and characterization could be suggested for future work since this compound was potential as antioxidant agent.

4. Conclusion

Ethyl acetate extract of *D. draco* resin provide higher antioxidant activity compare to methanol and *n*-hexane extracts. Fractionation of *n*-hexane fraction of ethyl acetate extract using column chromatography resulting fraction F.4 and F.5 which provide high antioxidant activity with IC_{50} value 14.19 µg/mL and 17.27 µg/mL, respectively. Separation of F.5 using preparative thin layer chromatography resulting two fractions (F.5.1 and F.5.2) with lower antioxidant activity compare to F.5. Characterization of fraction F.5.2 using infrared spectroscopy showed that component in fraction F.5.2 contains NH-substituted benzene.

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