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Antioxidant activity of Litsea petiolata Hk. f

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Abstract. Litsea petiolata Hk. f was included Lauraceae family, and the previous study had been isolated 5 compounds from the *Litsea petiolata* Hk. f stem bark dichloromethane extract namely harman or aribine, norharman, reticuline, isoboldine, and thalifoline. Antioxidants can prevent tissue damage by free radical. Free radical production continuously in all cells as cellular function usually, but excess production can cause many diseases. The research aimed to assay the activity of antioxidant from the extract and fractions of the Litsea petiolata Hk. f stem bark with DPPH assay and FRAP assay. The extract was obtained by soxhletation used dichloromethane as solvent. The fractions fractionated with column chromatography. The antioxidant test used DPPH assay and FRAP assay. The IC50 values for the 2, 2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging test of the dichloromethane extract was 27.36 µg/mL, the fraction A was 113.74 µg/mL, fraction B was 60.17 µg/mL, and the control positive (quercetin) was 3.96 µg/ml. The EC50 values for ferric ion reducing antioxidant potential (FRAP) test of the dichloromethane extract was obtained 13.47 μ g/mL, the fraction A was 76.49 μ g/mL, fraction B was 55.73 μ g/mL, and the control positive (quercetin) was 14.01 μ g/ml. The extract had higher antioxidant activity than the fractions, and by FRAP test the extract showed better antioxidant activity than the positive control (quercetin).

1. Introduction

Litsea petiolata Hk. f was included in Lauraceae family. Previous study had been isolated 5 compounds from the dichloromethane extract of Litsea petiolata Hk. f stem bark, namely harman or arhkibine, norharman, reticuline, isoboldine, and thalifoline [1].

Antioxidants can prevent tissue damage by scavenging the free radical agent. All cells in the body produce free radicals, but excess production can cause many diseases [2]. Many studies confirmed that antioxidants could prevent oxidation of cellular biomolecules because of prolonged exposure to Ultra Violet Radiation (UVR) on human skin [3]. UVR-induced damage involves depletion of endogenous antioxidants [3]. The result from previous research showed that many plant compounds could protect the skin from the negative impact of UVR [3]. This research objective was to know the antioxidant activity of *Litsea petiolata* Hk. f plant.

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2. Materials and methods

The *Litsea petiolata* Hk. f stem barks were collected from Hutan Sirnpan Rimba Teloi, Sik, Kedah, Malaysia by the Phytochemical group of the Chemistry Department, Faculty of Science, University of Malaya [1].

The extract was obtained by soxhletation after removed the lipid content from the stem barks dried powder with hexane for three days and moistened with NH₄OH [1]. The extraction used dichloromethane solvent for 18 hours [1]. The fractions were obtained with column chromatography and used two solvents, dichloromethane and methanol, by increasing polarity method.

The antioxidant activity was conducted using two assays namely FRAP (Ferric Reducing Antioxidant Power) assay and (2,2-Diphenyl-1Picrylhydrazyl) DPPH way [4]. Quercetin was used as the positive control.

The FRAP method was done by added 270 μ L FRAP reagent in 30 μ L of the sample solution in methanol at various concentrations. The FRAP reagent was prepared with 100 mL of acetate buffer pH 3,6 mixed to 10 mL of 10 mM TPTZ solution in 40 mM HCl and 10 mL of 20 mM FeCl, at a 96-well microplate and shaken up for a minute [5]. The mixtures incubation was done for 30 minutes at around 20–22 °C in the black. The absorbance was obtained using Versamax Elisa Microplate Reader (USA) at 593 nm. The antioxidant activity was performed in the following formula [5]:

This research was done triplicately. The blank was without sample. Inhibitory Concentration 50% (IC₅₀) was calculated by Software GraphPad Prism 7.0 [5].

The DPPH method was done by added 180 μ L of 150 mM DPPH solution in methanol, in 20 μ L of the sample or standard solution in methanol at a various concentration, at a 96-well microplate and shaken up for a minute. The mixtures incubation were done in the dark for 30 minutes at room temperature [6]. The absorbance was measured using Versamax ELISA Microplate Reader (USA) at 516 nm. The DPPH radical scavenging activity measuring used the following equation [5,6]:

% DPPH scavenged =
$$\frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100\%$$
 (2)

Where A sample was the absorbance of a test was sample at 517 nm after incubation for 30 minutes, a control is absorbance of 20 μ L DPPH in 180 μ L methanol at 517 nm after incubation for 30 minutes. Inhibitory Concentration 50% (IC₅₀) was calculated by Software GraphPad Prism 7.0 [5].

3. Results

The result of antioxidant activity from the extract, fraction A, fraction B, and quercetin used FRAP method can be looked at Table 1, Table 2, and Table 3.

Table 1. The inhibition value (%) of the samples from *Litsea petiolata* Hk. f stem bark dichloromethane extract used FRAP method triplicately.

Concentration	The inhibition value (%)		
(µg/mL)	Extract	Fraction B	
5.0	23.17 ± 2.41	4.10 ± 0.46	
10.0	47.63 ± 5.45	5.83 ± 0.90	
20.0	73.60 ± 5.64	18.40 ± 1.37	
40.0	97.73 ± 2.84	42.53 ± 0.70	
80.0	113.70 ± 4.45	68. <mark>70</mark> ± 2.75	
160. <mark>0</mark>	131.20 ± 1.13	103.13 ± 1.15	

Table 2. The inhibition value (%) of the fraction A from *Litsea petiolata* Hk. f stem bark dichloromethane extract used FRAP method triplicately.

Concentration (µg/mL)	The inhibition value of fraction A (%)
3.0	2.03 ± 0.32
6.0	5.63 ± 0.25
12.0	8.33 ± 0.25
24.0	16.10 ± 1.14
48.0	31.23 ± 0.93
96.0	62.77 1.43

Table 3. The inhibition value (%) of quercetin (positive control) used FRAP method triplicately.

Concentration (µg/mL)	The inhibition value of Quercetin (%)
3.0	12.43 ± 0.74
5.0	18.93 ± 1.14
7.0	26.63 ± 0.64
10.0	35.33 ± 0.75
15.0	53.73 ± 2.40

The result of antioxidant activity from the extract, fraction A, fraction B, and quercetin used DPPH method can be seen in Table 4, Table 5, and Table 6.

Table 4. The inhibition value (%) of the samples from *Litsea petiolata* Hk. f stem bark dichloromethane extract used DPPH method triplicately.

Concentration	The inhibition value (%)		
(µg/mL)	Extract	Fr <mark>ac</mark> tion B	
5.0	22.20 ± 1.83	5.19 ± 1.14	
10.0	29.20 ± 1.51	12.30 ± 2.38	
20.0	43.71 ± 3.34	26.86 ± 1.75	
40.0	63.99 ± 3.64	46.39 ± 2.55	
80.0	76.40 ± 1.32	63.34 ± 2.62	
160.0	82.87 ± 1.69	78.44 ± 0.71	

Table 5. The inhibition value (%) of the fraction A from *Litsea petiolata* Hk. f stem bark dichloromethane extract used DPPH method triplicately.

Concentration (µg/mL)	The inhibition value of fraction A (%)
3.0	-1.69 ± 1.51
6.0	2.27 ± 1.67
12.0	4.25 ± 0.27
24.0	8.80 ± 1.31
48.0	20.98 ± 3.67
96.0	42.02 ± 0.61

Table 6. The inhibition value (%) of quercetin (positive control) used DPP method triplicately.

Concentration (µg/mL)	The inhibition value of Q uercetin (%)
1.0	14.78 ± 1.73
2.0	29.61 ± 3.89
3.0	42.27 ± 3.10
5.0	60.78 ± 5.03
6.0	71.68 ± 6.94

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The result of calculated IC_{50} can be seen in Table 7.

Samples	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)	
	FKAF methou	DFF fi methou	
The Litsea petiolata Hk. f stem bark	13.47	27.36	
dichloromethane extract			
The fraction A of the extract	76.49	113.74	
The fraction B of the extract	55.73	60.17	
Quercetin	14.01	3.96	

Table 7.	The IC ₅₀	values	of the	samples.
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The other Litsea had been studied, namely *Litsea cubeba* from Bogor Indonesia, contained essential oil, laurotetanine, and phenanthrene and revealed potent antioxidant activity [7]. The other study of *Litsea cubeba* (Lour.) Heartwood ethanol extract showed that its chloroform fraction at pH 7 had IC₅₀ 23.81 \pm 0.01 µg/mL, and the isolate 3.12 \pm 0.02 µg/mL used DPPH method [8].

Litsea elliptica and *Litsea resinosa* methanol extract from the root and stem bark (from Sarawak, Malaysia) had antioxidant activity which is almost the same as hydroxytoluene butylated as the standard [9]. *Litsea garciae* (from Samarinda, East Kalimantan) contained total phenol 0.9-1.0 μ g/mg GAE and total flavonoid 10.1 μ g/mg CE [10]. The antioxidant activity of the ethyl acetate extract from *L. garciae* stem bark was 86% at 100 ppm concentration, with IC₅₀ at 41.54 ppm [10].

There was a study, that point out the correlation between the antioxidant activity used FRAP method with total phenolic contents (TPC) and complete alkaloid contents (TAC), and the antioxidant effect of alkaloids was higher than phenols [11]. These results showed that alkaloids and phenols were the essential substances for the antioxidation effect [11].

The result of the research presented that the IC_{50} value of the extract has IC_{50} was smaller than quercetin as control positive with FRAP method, so the extract potentially has antioxidant agent. The result of the DPPH assay showed that IC_{50} of the extract was higher than quercetin. The different effect was caused different the FRAP method mechanism with the DPPH method mechanism [12]. The FRAP method mechanism is electron transfer based, followed by a proton transfer [12]. The DPPH method mechanism is a hydrogen atom transfer from the phenolic OH group [12].

5. Conclusions

Antioxidant activity of *Litsea petiolata* Hk. f stem barks extract is higher than the fractions of the extract, and used FRAP method the extract has IC_{50} is lower than the positive control (quercetin), so the extract can be a potential antioxidant agent.

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