

NOTE

IRON CHELATING AND ANTIRADICAL ACTIVITY OF
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ABSTRACT

A methanol soluble fraction extracted from Kayu Manik leaves (*Trema orientalis*) from Seluma, Bengkulu, exhibited an antiradical activity 69.73% (scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical) that was almost similar to that of 1 mM ascorbic acid. On the other hand, the iron chelating activity was 40.74%. We believe that it would be useful to take the results as an alternative for processing industries and can be observed as a good source of new agent for iron chelator.

Keywords: Antiradical, Flavonoid, Iron chelating, Kayu Manik Leaves, Thalasemia

INTRODUCTION

Antiradical (antioxidant) is a species (compound or reagent) that act as a radical scavenger, inhibits lipid peroxidation and other free radical (including *Reactive Oxygen Species* - ROS) mediated processes [1-2]. It is already known that extracts from plants have been reported to demonstrated antioxidant activity by a variety of *in vitro* methodologies. There are many researches have shown that vegetables contain other antioxidant nutrients, in addition to well known vitamin C and E, and carotenoids. The presence of the antioxidant nutrients has significant contribution to their total antioxidant capacity. There are some evidences that vegetables are beneficial to health and contribute to the prevention of degenerative processes [3-4]. Therefore, some vegetables were selected to study their phenol and flavanoid contents and antiradical activities [1-7]. The results in almost cases confirm that vegetables has good antioxidant activities. The results provide evidences that some plants extracts could be used as antioxidant agent compared to synthetic antioxidant. For instance, Butylated Trihydroxy Toluene (BHT) are known as a synthetic antioxidant that has carcinogenic effects in living organism [5-8]. Antioxidants and other supportive therapies protect red blood cells (RBC) against oxidant damage [9]. The presence of ROS is also one of the characteristic of thalassemia diseases [10-12].

Humans body can not eliminate the iron resulted from the breakdown of the transfused red blood cells.

The excess iron is deposited as hemosiderin and ferritin in liver, spleen, endocrine organs and myocardium. The accumulation of iron in toxic quantities causes tissues damage and leads to complications such as heart failure, endocrine abnormalities like diabetes, hypothyroidism, liver failure and ultimately early death [10-11]. In this line, the transition metal ion such as Fe²⁺ possess the ability to move single electron even starting with relatively non-reactive radicals. The processes can allow the formation and propagation of many radical reactions [12].

To avoid ROS generation that associated with redox active metal catalysis involves chelating of metal ions in the human body. Iron chelators mobilize tissue iron by forming soluble and stable complexes, and then the complexes excreted in the feces and/or urine. Within this concept, there are many great efforts towards finding any potentially useful source to obtain the natural iron chelator and to get the optimum benefits [10-11]. Currently, two iron-chelating agents are licensed for the treatment of iron overload; deferoxamine and deferiprone. Although both of the iron chelating agents has clearly established, the prices are very expensive. The prices problem induces the inconvenient route of administration therapy, and it become as significant issue [13].

For thousand of years, alot of people has known about the benefit of drugs from nature. Plants extracts, for treatment for various deaseases, were highly

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regarded by the ancient civilizations. A medicinal plant, Kayu Manik Leaves (*Trema orientalis*) that traditionally used for management of diseases of Rejang ethnic in Province of Bengkulu (Indonesia) was selected as materials for this study. There are some studies on the utility of this plant, for example, the leaves are used to treat coughs, sore throats, dysenteries and hypertension and the bark is used to make a cough syrup, [14]. In Rejang ethnic the *Trema orientalis* leaves have been used for stomach ache and diarrhea treatments. Based on the present evidences, we are interested to explore the other functionality of the plant for other possibilities. Here, we report our investigation toward some aspects *i.e.*: flavonoid content, antiradical activity toward DPPH and iron chelating activity.

EXPERIMENTAL SECTION

Materials

Fresh leaves of Kayu Manik were collected from Seluma, Bengkulu. Gallic acid, quercetin, Na-EDTA, methanol, ethanol, potassium acetate, aluminium chloride, acetic acid, chloroform, DPPH, Ascorbic Acid, potassium ferricyanide, ferric chloride, ferrozine solution were purchased from Sigma Aldrich and used as received. Distilled water was purchased from Laboratory of Chemistry, Faculty of Mathematics and Natural Science, University Bengkulu (Unib).

Instrumentation

Visible Spectrophotometer, Balance, Rotary Evaporator, glass apparatus, pH meter was available at Laboratory of Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Science, University of Bengkulu (Unib).

Procedure

Preparation of methanol extracts

The air-dried and finely ground samples were extracted by using the maceration method and methanol was used as solvent. The extract was filtered and concentrated in vacuo at 40 °C, yielding a semi-solid material and kept in dark at about 4 °C until tested.

Flavonoid Contents

Flavonoid content was determined by following colorimetric method [15]. Briefly, 0.5 mL of plant extract (1% w/v) in methanol was separately mixed with 1.5 mL of methanol 0.1 mL of 10% aluminium chlorida, 0.1 M of potassium acetate, and 2.8 mL of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with visible

spectrophotometer. The calibration curve was constructed by preparing quercetin solutions at concentration 0 to 150 mg L⁻¹ in methanol.

Assay toward DPPH radicals

The effect of DPPH radical was evaluated by the method of Okada [16] with a slight modification. The assay mixture contains 300 μ L of 1.0 mM DPPH solution, 2.4 mL of 99% ethanol and 300 μ L of sample solution. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid (0.1 and 1.0 mM) was used as positive control.

Iron Chelating Activity

The chelation of ferrous ions by extracts was estimated by method of Dinis [17]. Briefly, 50 μ L of 2 mM FeCl₂ was added to 1 mL of the extracts (1% b/v). The reaction was initiated by the addition of 0.2 mL of ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine-Fe²⁺ complex formation was calculated using $[(A_o - A_s)/A_o] \times 100$, where A_o is the absorbance of the control and A_s is the absorbance of the extract/standard. Na₂EDTA was used as standard.

RESULT AND DISCUSSION

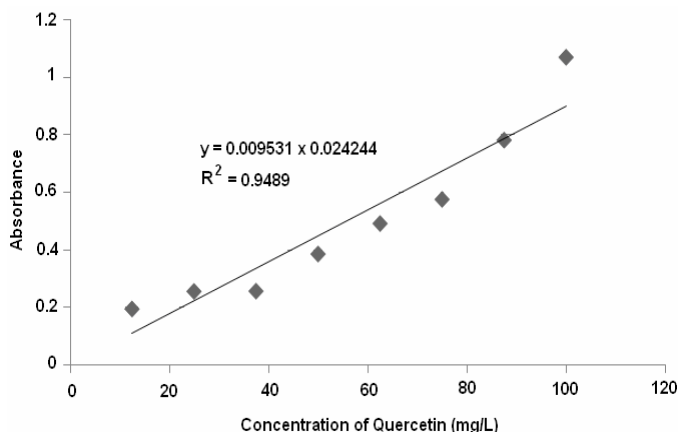
The extract obtained by maceration method using methanol as solvent was screened for their possible antiradical (antioxidant) activity, namely DPPH free radical scavenging system. Beside that, the material was also tested in iron chelating activity system. The use of methanol for extraction caused by the ability of the solvent to solve/extract the polar compounds from the sample such as flavonoid and phenolic compounds.

Flavonoid Contents

Flavonoid was widely accepted as a compound which has antioxidant activity. They have some effects on human nutrition and health. Food-derived flavonoid such as flavonols quercetin, kaemferol, and myricitin has been reported to reduce cancer risk [5]. Beside that, flavonol are known act as metal chelators [18]. Based on the current literature that the flavonoids are responsible for the antioxidation agent in the natural products [4], hence in our study, the flavonoid contents of Kayu Manik leaves (*Trema orientalis*) from Seluma, Bengkulu was determined and expressed as quercetin equivalent. The calibration curve of quercetin standard

Table 1. Flavonoid Content of Kayu Manik Leaves 1% (b/v)

Sample	A	C (mg/L)	\bar{C} (mg/L)	Dilution factor	\bar{C} (mg/L)
<i>Trema orientalis</i> leaves from Seluma, Bengkulu	0.069	9.9715			
	0.072	10.2924	10.64883	100 x	1064.883
	0.085	11.6826			

**Fig 1.** Calibration Curve of Quercetin

is shown in Fig. 1.

Based on the calibration curve (Fig. 1) and the absorbance of the extract (Table 1), we found that the flavonoid content of *Trema orientalis* leaves extracts (1% b/v) was 1064.883 mg/L (Table 1).

Assay toward DPPH radicals

Free radicals play an important role in the origin of life and biological evolution. The radicals have several beneficial effects on organism [1-2]. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is free radical compound and has been widely used to test the free radical-scavenging ability of various samples [3-4]. To evaluate the scavenging effect of DPPH on the extracts from Kayu Manik leaves from Seluma, Bengkulu, DPPH inhibition was investigated and the result is shown as relative activity against control (eq. 1).

$$\% \text{ Activity} = \left(1 - \frac{\text{Absorbance at 520 nm in the presence of sample}}{\text{Absorbance at 520 nm in the absence of sample}} \right) \times 100 \quad (1)$$

therefore,

$$\% \text{ Activity} = \left(1 - \frac{0.088}{0.293} \right) \times 100 = 69.96\% \quad (2)$$

The experiment was repeated three times and gave the activity, 69.96%, 70.99%, and 68.26%, respectively or the average of the activity was 69.73%. The activity of the extract is higher than the activity of ascorbic acid 0.1 mM (the % activity is 45.39%) and closer to the activity of ascorbic acid 1 mM (the % activity is 92.21%). This result confirms that the *Trema orientalis* leaves extract from Seluma, Bengkulu, has moderate antioxidant activity. There is one report on the

isolation of eight compounds from the MeOH extract of the stem of *T. orientalis* which collected from China; (-)-epicatechin, (+)-catechin, (+)-syringaresinol, trans-4-hydroxy-cinnamic acid, (-)-ampelopsin F, *N*-(trans-*p*-coumaroyl)tyramine, *N*-(trans-*p*-coumaroyl)-octopamin, and 3,5-dimethoxy-4-hydroxyphenyl-1, *O*- β -D-glucoside [20]. Although the what kind of the active compound and mechanism of antiradical activity is not fully understood, some explanations have been provided for antioxidant activity. The sequestration of free radicals; hydrogen donation; metallic ion chelation by synergistic works of the compounds are believed play an important role in the activities [20-22].

Iron Chelating Activity

In general some ligands can form complexes compound with Fe^{2+} . There are some known ligand having nitrogen electron donating atom such as pyridine, 2,2-bipyridine, and 4,4-bipyridine and ferrozine. The ferrozine can quantitatively form complexes with Fe^{2+} [16,23-24]. However in the presence of stronger ligand such as EDTA as a chelating agent, the complex formation is disrupted and physically could be identified with the decreasing of red color of the complex [23-24]. Based on the experiment result, using the eq. 3.

$$\% \text{ Inhibition} = \left(\frac{A_0 - A_s}{A_0} \right) \times 100\% \quad (3)$$

where : A_0 = Absorbance in the absence of sample

A_s = Absorbance in the presence of sample

Therefore,

$$\% \text{ Inhibition} = \left(\frac{0.092 - 0.049}{0.092} \right) \times 100 = 40.74\% \quad (4)$$

We found that methanol extract of *Trema orientalis* leaves from Seluma, Bengkulu, has iron chelating activity 40.74%, indicating the activity was about a half of the activity of EDTA ligand. EDTA (1% b/v) was used as control in this experiment having chelating activity 93.15%. This result clearly shows that the extract has a challenge to use as an iron chelating agent, although there are some clinical experiments are needed to prove it could be used *in vivo* manner. [25].

CONCLUSION

In summary, *Trema orientalis* leaves (methanol extract) from Seluma, Bengkulu, has flavonoid content and good iron chelating and antiradical activities. The iron chelating activity provide evident that the extract could be used as a potential for herbal iron chelator.

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