ANTIOXIDANT AND TOXICITY ACTIVITIES OF EXTRACTS FROM Koordersiodendron pinnatum Merr. (ANACARDIACEAE) LEAVES

Megawati, Sofa Fajriah, M. Hanafi, Puspa D.N.L, A.Darmawan, Salahuddin

Research Center for Chemistry, Indonesian Institute of Sciences,
Kawasan PUSPIPTEK Serpong, Tangerang Banten 15314
Email :ga_lipi@yahoo.com

Abstract

Research on the chemical content and activity of Koordersiodendron pinnatum Merr.(Anacardiaceae) plant has no publication, so this research on the antioxidant activity and bioactivity screening using BSLT (Brine Shrimp Toxicity Lethally) method had been done. An extract is active as an antioxidant when it has IC50 values <200 µg/mL and screening bioactivity (BSLT) is said active if it has LC50 values <250 µg/mL. Based on these criteria, the results of anti-oxidants from methanol extract, n-hexane fraction, ethyl acetate fraction and the residue of K. pinnatumleaves have IC50 45.33 ;> 1000; 89.40 and 14.29 µg/mLand with LC50 values are 1000; 243 ; 400 and > 1000 µg/mL, respectively. From these data, the residue of K. pinnatum leaves has potential as antioxidants with IC50 14.29 µg/mL and n-hexane fraction has the highest bioactivity against shrimp larvae Artemia salina with LC50 243 µg/mL .

Keywords : *Koordersiodendron pinnatum Merr., Anacardiaceae, antioxidant, BSLT*

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INTRODUCTION

Koordersiodendron pinnatum plant is a large deciduous tree to 40 m high; stem to 80 cm in diameter, with short rounded buttresses; outer bark dark brown or blackish, fissured; inner bark laminate, fibrous, pink to red.Its leaves is odd-pinnate, spirally arranged, often crowded at end of twigs, 50-80 cm long; leaflets 10-16 pairs, ovate-oblong to narrowly oblong, sometimes subfalcate; the basal ones smaller; base obtuse to oblique; apex acuminate to subcaudate; glossy dark green above, paler or golden brown beneath; the margin wavy.This plant has flowers white, in axillary racemes to 50 cm long, arising from among the foliage.It is widely distributed throughout Philippines, Borneo, Sulawesi, Moluccas, and Papua New Guinea; in lowland forests (Lemmens *et al*., 1995).

Antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats, due to the lipids peroxidation. As a consequence increases food deterioration, discoloration, and nutritional losses, among others. In order to stop these deterioration processes the addition of synthetic antioxidants named butylatedhy droxyanisole (BHA), butylatedhydroxytoluene (BHT), tertiary butylhydroquinone, and propyl-gallate has been widely used industrially. The ability of phenolic substances including flavonoids and phenolic acids acting as antioxidants has been reported [Liu, Y.W *et al* .,2003]. Tannins have been reported to have strong antioxidant activity [Cai, Y.Z *et al*.,2006]. There is also growing interest both in industry and in scientific research in spices and medicinal herbs because of their antimicrobial and antioxidant activity [Eyob, S., *et al*., 2008].

Antioxidants are compounds that can inhibit the rate of oxidation or neutralize free radicals. Anti-oxidants are substances that can neutralize or destroy free radicals. Free radicals are oxygen species that have high levels of reactive and occurs naturally in the body as a result of biochemical reactions/metabolism. Free radicals are also present in the environment around us that could come from air pollution, tobacco smoke, excessive evaporation of alcohol, preservatives and fertilizers, Ultra Violet rays, X-rays, and ozone. Free radicals can damage cells when the body lacks an anti-oxidant or when the body excess of free radicals. This can lead to the development of cancer cells, liver disease, arthritis, cataracts, and other degenerative diseases, and even accelerate the aging process (Yang, J(2002). Free radicals can damage cell membranes and change DNA sequences (mutation). Mutations may increase the risk of cancer and damage to and deactivate the protein. Existing the antioxidant compounds that can be measured the potent using DPPH method could minimized amount of our body free radicals, while the reactions that occur of DPPH method (Yen G et al.,1995).

Bioactive compounds in high doses are (almost) always toxic. Thus, *in vitro* killing power of the compounds against the animal organism can be used to screening plant extracts which has bioactivity, and to monitor bioactive fraction during the fractionation and purification. One of organism suitable for toxicity animal testing is a brine shrimp (crayfish) (B.N. Meyer. *et al*,1982). To determine toxicity and antioxidant activity of leaf extract *Koorsidendronpinnatum*, we used BSLT (*Brine Shrimp Lethality Test*) method and DPPH (*-1,1-diphenyl-2-pikrilhidrazil*) method.

This study was limited to testing the *K. pinnatum*crude extract of each solvent used. Measurement of antioxidants through reduction of free radical DPPH is a simple, fast method and did not required a lot of reagents as well as other tests (xanthine-xanthineoxidase, thiocyanate method, total antioxidant).

The measurement results indicate the level of toxicity and potential antioxidant activity of the samples in general. In the second method, *brine shrimp* serves as animal tester to determine toxicity levels of the extract, that calculated based on the number of dead shrimp larvae correlated with the extract concentration used. While, in the antioxidant activity test method, DPPH acts as free radical reduced by

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antioxidants compounds came from samples, which DPPH reacts with antioxidants to change DPPH into *1.1,-diphenyl-2-pikril hydrazine*. This reaction causes the color change that can be measured with visible light spectrophotometer at λ 515 nm, so the reduction activity of free radicals by the sample can be determined (Juniarti *et al*., 2009).

MATERIAL AND METHODS

Plants Material

Koordersiodendron pinnatum (Anocardiaceae) was collected from Mekongga forest, South at Sulawesi at November 2010. A voucher specimen has been deposited in the Herbarium Bogorienses, Indonesia

Equipments

Equipments used in this study are glassware, tools maceration and Rotavapor (Buchi R214-Switzerland), micro pipette (SOCOREX, Switzerland), electric ovens, UV-Visspectrometer (Hitachi U-2000, Series No. 0372-026).

Chemicals

The chemicals used n-hexane and methanol technis for extraction and methanol pa. (E. Merck) for DPPH solvent, DPPH (1,1-diphenyl-2-pikrihidrazil) (Aldrich), and quercetinfor positive standard.

Preparation of Herbal Extracts

The air-dried aerial parts of the plants were cut or milled into small pieces (200 g) and extracted with n-hexane (200 ml) and methanol (200 ml) at RT for 3 times. The combined n-hexane and methanol extracts were evaporated under vacuum, to give crude n-hexane and methanol. The crude methanol extracts were fractination with solvent n-hexane, ethyl acetate andresidu. residu fraction at coloumn chromatografhy than tested for the antioxidant activity dan bioactivity (BSLT).

DPPH radical scavenging method (Artanti *et al*., 2003)

Antioxidant analysis was conducted using modification of "DPPH free scavenging activity" method. Various concentrations of the mistletoe extract/fractions in 0.8 ml methanol were mixed with 0.2 ml of methanolic solution containing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, resulting in a final concentration of the DPPH of 0.2 mM and sample concentrations up to 100 µg/mL. The mixture was shaken vigorously and left to stand for 30 min in room temperature, the absorbance was then measured using spectrophotometer at 515 nm. Percentage of inhibition (free radical scavenging activity) was calculated by the equation: [1 - (B/A)] x 100%; whereas A is absorbance in the absence of sample and B is absorbance in the presence of sample. IC50 value denote the concentration of sample required to scavenge 50% DPPH free radicals.

Toxicity Test Method BSLT (Meyer's et al.,1982). used to study the general toxicity of samples using shrimp eggs (Artemiasalina Leach). Two vessels prepared for shrimp hatcheries and for larvae. In one vessel in the vessel was placed lamp to warm temperatures in the hatchery, while in the other vessel next to the sea water was not given. Into the sea water entered ± 50-100 mg shrimp eggs for hatching, incubated for 48 hours. Extracts to be tested are made in a concentration of 10, 100.500 and 1000 ppm in sea water. If the sample does not dissolve, add 10 mL of DMSO. Procedure: About 100 mL of sea water containing 10-11 larvae of the shrimp, inserted into the test container. Added sample solution to be tested 100 mL each, with concentrations of 10, 100, 500 and 1000 ppm, respectively. Each concentration was carried out three repetitions (triplicate). Solution was stirred until homogeneous. To control is done by adding 10 mL of DMSO. Solution was left for 24 hours, counted the dead and live number of larvae in every hole. Amount of died larvae number summing the dead larvae that died in each concentration (3 holes), the same way to count the live larvae number based on the life larvae in

each concentrations. Accumulated dead number calculation was carried out in the following way: the accumulation of dead to 10 ppm = number dead at these concentrations, the accumulation of dead to 100 ppm concentration = number of dead at 10 ppm + number of dead at 100 ppm concentration, accumulation of dead to 500 ppm concentration = number of dead at 10 ppm + number dead at concentrations of 100 ppm + number dead at a concentration of 500 ppm. Accumulated number of dead counted up to 1000 ppm. Calculation of accumulated life of each concentration was carried out in the following manner: accumulation of life to 1000 ppm = number living at 1000 ppm, the accumulation of life for the concentration of 500 ppm = number living at 1000 ppm + number of living at a concentration of 500 ppm, the accumulation of life for the concentration of 100 ppm = number living at 1000 ppm + figures life at a concentration of 500 ppm + number of living at a concentration of 100 ppm. Accumulated number of life calculated to 10 ppm. Furthermore, mortality is calculated by: the accumulation of dead divided by the accumulation of life and death (total) multiplied by 100%. Graphics made with the log concentration as the x-axis on mortality as the y-axis. LC50 value is the concentration of a substance which causes the death of 50% obtained by using linear regression equation y = a + bx. An active or toxic substance to say when the value of LC50 <1000 ppm to extract and<30 ppm for a compound. Another criteria activity of Crude extracts resulting in LC50 values of less than 250 g/ml were considered significantly active and had the potential for futher investigation (Rieser et al., 1996).

RESULT AND DISCUSSION

The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to be decolorized in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance.

In the quantitative assay the extract exhibited a notable dose dependent inhibition of the DPPH activity, with a 50% inhibition (IC_{50}), was summarizes in Table I. The result of antioxidant indicated showed with DPPH method, where the presence of DPPH compounds, capable of inhibiting free radicals present in the sample to be tested, it showed antioxidant activity from partition of *Koordersiodendronpinnatum (Anacardiaceae)* methanol extract, shown in Table I.

Table I. Antioxidant Activities (IC50) of fractination form methanol extract of *Koordersiodendron pinnatum (Anacardiaceae)* leaves

No	Sample	IC_{50} (µg/ml)
1	Quercetin (standard)	2.71
2	Methanol extract	45.33
3	Hexane fraction	>1000
4	Ethyl acetate fraction	89.40
5	Residue fraction	14.29

From these date, it shows that residue fraction has the highest activity as antioxidant with IC_{50} 14.29 ug/mL. The results revealed that the active principles were mainly distributed in polar fraction Because of that, the active fraction of residue of *K. pinnatum* leaves further purify using vacuum liquid chromatography and the result of antioxidant activity can be seen at Table 2. In this results also polar fraction shown potential antioxidant activity, indicated at F10. with IC_{50} value is 46 µg/ml.

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Table II. Antioxidant Activities from coloumn chromatography of residue fraction from *Koordersiodendronpinnatum (Anacardiaceae)* leaves

No	Sample	IC_{50} (mg/ml)
1	Quercetin (standard)	2.71
2	F1	> 1000
3	F2	713
4	F3	> 1000
5	F4	>1000
6	F5	726
7	F6	197
8	F7	177
9	F8	55
10	F9	106
11	F10	46

From bioactivity screening result using BSLT method from *K. pinnatum* leaves extract (Figure 1), it showed that the *n*-hexane fraction has the highest bioactivity against shrimp larvae.

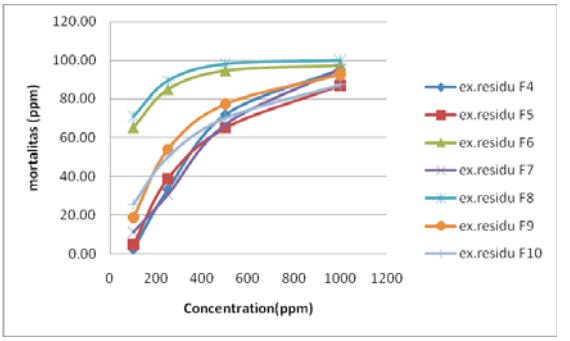

Figure 2.Bioactivity test from coloumn chromatography of residu fractination *Koordersiodendronpinnatum (Anacardiaceae)*

The bioactivity screening result of residue fraction of *K. pinnatum* leaves can be seen at Figure 2. From this figure, the residue fraction of F-8 has the highest bioactivity against shrimp larvae.

Based on this results, therefore, further isolation of the highly active fractions may lead to the discovery of new cytotoxic compounds and structure elucidation from this plant.

Conclusion

The residue of *K. pinnatum* leaves has potential as antioxidants with IC50 14.29µg/mL and n-hexane fraction has the highest bioactivity against shrimp larvae with LC50 243 µg/mL.

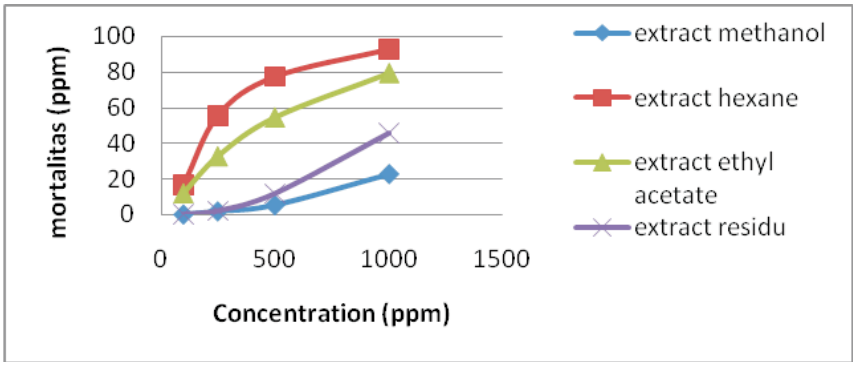

Figure 1.Bioactivity test (BSLT) the results of extract methanol, hexane, ethyl acetate and residue

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