CYTOTOXIC, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF *Xylocarpus moluccensis* FRUIT HUSK

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Abstract

The aims of this study were to examine toxicity, antioxidant and antibacterial activity of methanol extract of fruit husk (Xylocarpus moluccensis). Toxicity assay was undertaken by brine shrimp lethal toxicity test, antioxidant activity was done by Diphenyl picryl hydrazil (DPPH) scavenging, and inhibition growth to Staphylococcus aureus and Escherichia coli used as antimicrobial test. The results showed that methanol extract of fruit husk was not toxic, antiradical efficiency was 0.000154, and broad spectrum antibacterial with inhibition zone to Staphylococcus aureus and Escherichia coli was 12.7±1.2 and 11.9±0.9 mm, respectively in 630000 ppm concentration.

Key words : Cytotoxicity, antioxidant, antibacterial, methanol extract, fruit husk, *Xylocarpus moluccensis*

1. Introduction

The *Xylocarpus moluccensis* belongs to the order Geraniales of the family meliaceae The genus Xylocarpus is distributed in the coastal regions of India, Ceylon, Burma and Malaya [1] and Indonesia. The fruit of *Xylocarpus moluccensis* is a green color, lemon fruit sized, hard and heavy, leading to the common name 'cannon ball tree'.

This mangrove provides a several phytochemical compounds. It can be used for health therapy. Several biological properties have been attributed to *Xylocarpus moluccensis*: aphrodisiac, fever, malaria, hair preservatives, astringent, antidiarrhoea, antiemetic and haemostatic properties [2,3]. In Indonesia, the use of medicinal plants such as mangrove to cure several illnesses has been use routinely by coastal native. Furthermore, Porong estuary is rich in various mangrove species with diverse biological and pharmacological properties.

The objectives of this work were to proceed to the preliminary cytotoxic, antioxidant and antibacterial assessment of *Xylocarpus moluccensis* fruit husk.

2. Experimental Details

Plant material

The fruit of mangrove *Xylocarpus moluccensis* was collected from Porong's river estuary. The fruits of *Xylocarpus moluccensis* then were packed in Poly ethylene plastic and eventually brought to laboratory. The fruit was pelled to obtain the husk. Afterward, the husk was dried until ± 15% water content in a husk.

Extraction

The *Xylocarpus moluccensis* fruit husk was extracted by maceration method. The 25 g dried fruit husk was soaked in 75 ml methanol in an erlenmeyer for 24 hour. The extract was then separated from the debris by filtration with a Whatman no. 1, afterward the extract was concentrated using rotary vacuum evaporator. The extract then diluted to obtain several concentration of crude methanol extract. The methanol extract were subjected for bioactivity studies.

Antibacterial assay

The antibacterial activity test was done by paper disc diffusion method [4]. The microorganisms used in this study were *Staphylococcus aureus* and *Eschericia coli*. The 10^8 cfu/ml each inoculums were seeded in Mueller Hinton Agar (MHA, Oxoid) using sterile cotton swab. Sterile 6 mm paper blank discs were impregnated with different concentration of crude extract and

plated onto seeded Petri disc. The plates then were incubated in 35^0C for 24 hour. The inhibition zone around each disc was measured in millimeter.

Antioxidant assay
The antioxidant was assayed using DPPH radical-scavenging activity method following [5], 1 ml Different concentrations of crude methanol extract was added to 3 ml 0.5 mM DPPH solution (0.2 mM in methanol) as free radical source. The Scavenging activity was measured spectrophotometrically. The absorbances were recorded after 20 min at room temperature. . The disappearance of DPPH was read spectrophotometrically at 515 nm. Radical Scavenging Activity (RSA) was calculated. From the obtained RSA values, the EC_{50} were calculated, which represents the concentration of the scavenging compound that caused 50% neutralization.

$$RSA\ (\%) = \frac{A_0 - A_{30}}{A_0} \times 100$$

RSA = Radical Scavenging activity
A_0 = the absorbance at 0 min test
A_{30} = the absorbance at 30 min test

Antiradical Efficiency
Antiradical efficiency (AE) is a new concept of antiradical efficiency which combines the factors EC_{50} and T_{EC50} was defined as AE. It was calculated following [6] method:

$$AE = \frac{1}{EC_{50} \times T_{EC50}}$$

AE = Antiradical efficiency
EC_{50} = Effectives concentration
T_{EC50} = the time needed to reach the steady state to EC_{50}

Cytotoxix assay
The cytotoxic of methanol extract of fruit husk was assayed using brine shrimp lethally test (BSLT), The *Artemia salina* was prepared in which hatched eggs (obtained from Laboratory of Fish Breeding, Faculty of fisheries and marine science) in 2 day lighted sea water . The assay was prepared with 3 ml of filtered sea water containing 10 free swimming nauplii of *Artemia salina* in cavity bottle. 1 ml of variably crude extract concentration was plugged in cavity bottle for 24 hour. The percentage of mortality was determined by comparing the mean surviving *Artemia salina*. IC_{50} was determined using probit analysis [7]. Extracts giving LC_{50} values greater than 1000 ppm were considered to be nontoxic.

Data analysis
All data were calculated using the Microsoft Excel computer program.

3. Result and Discussion

***Xylocarpus moluccensis* fruit husk extract**
The extraction of *X. moluccensis* fruit husk resulted yield of 0,82 % from dried weight. Yellowish extract was obtained from fruit husk (Figure 1). Analysis phytochemical revealed that fruit husk contained flavonoid, and alkaloid (data not shown).

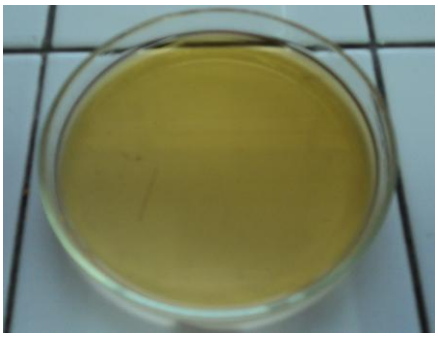

Figure 1. The extract of *X. moluccensis* fruit husk

Antibacterial assay
The antibacterial assay of methanol extract result, showed in Table 1. The extract had low in vitro potential of antimicrobial activities against *Staphylococcus aureus* and *Eschericia coli*. The maximum activity of methanol extract of *Xylocarpus moluccensis* fruit husk in S.aureus was observed against *S.aureus* (12.7±1.2 mm). Resemble result also was showed in *E. coli.* The maximum activity of methanol extract of *Xylocarpus moluccensis* fruit husk in *E. coli* is (12.7±1.2 mm) in the same concentration.

Tabel 1. Antibacterial activity of the methanolic extract of *Xylocarpus moluccensis* fruit husk in *S. aureus* against the test organisms.

Concentration (ppm)	zone inhibition (mm)	
	S.aureus	*E. coli*
630000	12.7±1.2	11.9±0.9
63000	8.4±0.4	7.1±0.9
6300	8.1±0.3	6.9±0.6
630	7.6±0.3	6.6±0.9
63	7.5±0.3	6.6±0.4

6.3	7.4±0	-

Based on the color of and phytochemical screening, another antibacterial compounds that might be exist in fruit husk of *X. moluccensis* are flavonoid and alkaloid and this is probably the natural defense against microorganism contamination in *Xylocarpus* sp. seed. [8] found that *Xylocarpus* sp. contained N-Methylflindersine that was known as antibacterial compound. Catechin, The bioactive compound that can inhibit the growth of lactic acid bacteria also was found by [9].

Antioxidant assay

The Radical scavenging activity of fruit husk methanol extract show in Figure 2.

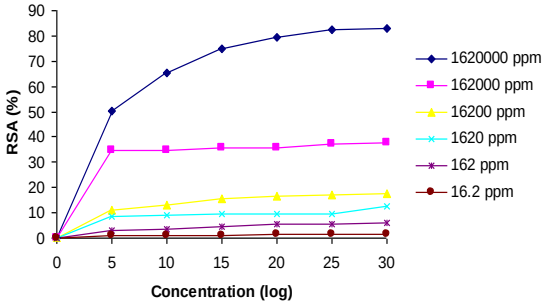

Figure 2. Radical Scavenging Activity of the methanol extract of *Xylocarpus moluccensis* fruit husk

The EC_{50} value of methanol extract of *X. moluccensis* fruit husk is 217000 ppm. This result agrees with previous result on the low antioxidant activity of *X. moluccensis* fruit peel. Considering it is still a crude extract. Therefore, *X. moluccensis* fruit husk components may be used as potential natural antioxidants in foods as well as health-promoting substances.

Antiradical efficiency

The antiradical efficiency was used to calculate the effectives scavenging of atiradical. In this calculation considering the time to reach until steady state. Based on the regression value (Figure 3). The AE of the crude methanolic extract of *X. moluccensis* of fruit husk is 0.000154.

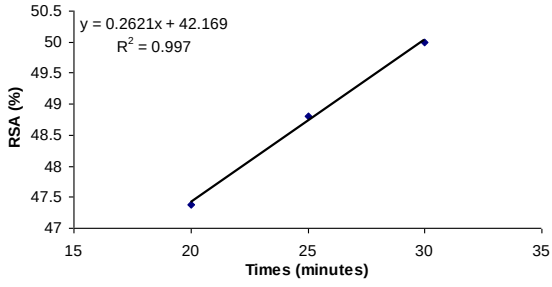

Figure 3. Regression of Antiradical efficiency of *X. moluccensis* fruit husk methanolic extract.

According the classification of AE by [6], the crude methanolic extract of *X. moluccensis* of fruit husk has a low level antiradical efficiency. We proposed because the fruit husk of *X. moluccensis* is not designed to muffle radical.

Cytotoxic assay

The results of the cytotoxicity assay against brine shrimp of the methanol extract of *Xylocarpus moluccensis* fruit husk are shown in Figures 3. The methanol extract of *Xylocarpus moluccensis* fruit husk has no significant cytotoxicity activity against brine shrimp with an LC_{50} value of 2.0 x 10^5 ppm. Based on [10], the result exposed that the methanol extract of *Xylocarpus moluccensis* fruit husk might be has no potency to be used in cancer cell line therapy but this signified that it might not be toxic to human.

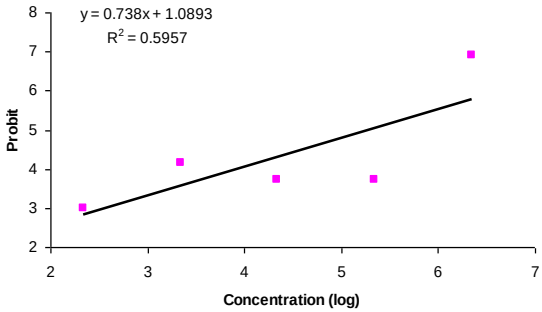

Figure 4. The toxicity effects of the methanol extract of *Xylocarpus moluccensis* fruit husk using brine shrimp lethality assay

The same result was exposed by [11] that *Xylocarpus* sp. did not show cytotoxic activity when it is extracted using polar solvent.

4. Conclusion

The result of this research indicates that methanol extract of of *Xylocarpus moluccensis* fruit husk showed a broad spectrum against gram positif and gram negative bacteria, low antioxidant activity and also showed non toxic activity.

5. Acknowledgements

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