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Toxicity Assay of Fermented *Artocarpus altilis* Leaves Using Brine Shrimp Lethality Test

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Abstract

Fermented *Artocarpus altilis* leaves contains phenolic compound that has pharmacological activity. Differences in fermentation method, *i.e.,* aerobic and anaerobic fermentation might change its characteristics and biological activity. The purpose of this study was to compare toxicity properties between aerobically and anaerobically fermented *Artocarpus altilis* leaves using brine shrimp lethality (BSL) test. Both types of fermented leaves were cold extracted using ethanol solvent (1:6) for 3 x 24 hours. Phytochemical screening was then performed to examine the presence of secondary metabolite compounds. BSL test was performed in 7 treatment groups, *i.e.,* negative control, 1000, 500, 100, 50, 25, 12.5 ppm of extract. Each vial contained 10 Artemia larvae, extract, and sea water up to 10 ml. Toxicity is measured by calculating LC_{50} after 24 hours observation. The experiment was repeated three times. The results of the study showed that LC_{50} of aerobic extract was 712 ppm, thus it can be categorized as toxic, while that of anaerobic extract is 1.927 ppm, or non-toxic to the *Artemia salina* larvae. In conclusion, aerobically fermented *Artocarpus altilis* leaves had toxicity potency, while anaerobically fermented *Artocarpus altilis* leaves had no toxicity potency against *Artemia salina* in BSL test.

Key words: *Artocarpus altilis,* brine shrimp lethality test, toxicity.

Introduction

Artocarpus altilis is one of the Indonesian plant that is commonly used as medicinal plants. It was empirically used to treat kidney disease, liver disease, high blood pressure, diabetes, *etc*. It contains phenol and quercetin that were considered has pharmacological activities.[1,2] The *Artocarpus altilis* leaves are oval with tapered ends. The edges of *Artocarpus altilis* vary, some are slightly grooved, not deeply curved, and grooved in. The size is quite large with a length of 30-60 cm and 20-40 cm wide. The color of the leaves on the top of the leaves is shiny green with smooth surface. Generally, the leaves grow flat and face upwards. The distance between the leaves varies between 2-10 cm.[3] Despite its widespread use as medicinal plant, there is limited information on toxicity of this plant.

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Table 1. Characterization of *Artocarpus altilis*

Characteristics	Aerob Fermentation	Anaerob Fermentation
Water content	12.0 %	18.0 %
Ash content	13.5 %	20.5 %
Ethanol soluble content	12.0 %	13.0%
Water soluble content	18.0 %	23.0%
Dried percentage	12.2 %	12.2 %

BSL test is one of the toxicity test method that is widely used in screening of bioactive compounds that are toxic from natural materials. BSL test is perfromed on *Artemia salina* larvae.[4,5]

Fermentation is defined as a process of energy formation through the catabolism of organic compounds. The industrial microbiology defines fermentation as a process of utilization of microbes to produce products.[6] Previous study showed that secondary metabolites in aerobically fermented *Artocarpus altilis* are saponin, alkaloid, flavonoid, tannin and saponin, while those of anaerobically fermented leaves included flavonoids, tannins and quinon. Fermentation process affected the composition and biological activity of the plant extract.[6,7]

Since there is only few studies investigating toxicity properties of *Artocarpus altilis,* this study was conducted to compare toxicity properties between aerobically and anaerobically fermented *Artocarpus altilis* leaves using BSL test.

Methods

Materials and tools

Materials used in this study included fresh *Artocarpus altilis* leaves obtained from Banjaran, Bandung, West Java; *Artemia salina* larvae, distilled water, ethanol, and sea water. Plant determination was conducted at the Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjajaran University, Indonesia. The tools used in this study were analytical balance, oven, aerator, incandescent bulb, loupe, and other laboratory glassware.

Extraction

About 80 sheets of *Artocarpus altilis* leaves were cleaned and fermented in 2 methods,

Table 2. Result of Phytochemical Screening

Substances	Plant		Extract	
	Aerob	Anaerob	Aerob	Anaerob
Alkaloid	+	-	+	-
Flavonoid	+	+	+	+
Tannin	-	-	-	-
Monoterpen, Sesquiterpen	-	-	-	-
Triterpenoid & Steroid	+	+	+	+
Quinon	+	+	+	+
Saponin	+	+	+	+
Phenolic	+	+	+	+

Table 3. Result of BSL Test

Extract	Repetition	Concentration (ppm) / Dead Larvae						Initial Amount of Larvae
		12.5	25	50	100	500	1000	
Aerob	1	0	0	1	1	2	10	10
	2	0	0	1	2	1	9	10
	3	0	1	1	1	1	6	10
Anaerob	1	0	0	1	1	2	5	10
	2	1	2	2	1	3	3	10
	3	0	3	2	2	2	0	10

i.e., aerob and anaerob fermentation. Around 300 g of the plant were cold extracted using ethanol solvent (1: 6) or until all soaked for 3x24 hours. Solvent is renew in every 24 hours. The extract was then evaporated using rotary evaporator 40 ^{0}C to produce viscous extract. Phytochemical screening was then performed to detect the presence of alkaloids, flavonoids, tanin, phenolate, monoterpene and sesquiterpene, steroids and triterpenoids, quinon, and saponin. Besides, ash content, and water soluble content were also evaluated.

BSL Test

Hatching of *Artemia salina* eggs was performed using sea water media. The hatching container is divided into two parts, *i.e.,* the light and the dark part. The perforated beam became the path for the born larvae to move naturally toward the light. During hatching, the hatchery is illuminated with 25-40 watt incandescent light (25℃ - 30℃). The media was aerated to ensure balance air circulation. After 48 hours, the larvae was ready to be used in the BSL assay.

The main solution (2000 ppm) was made by dissolving 50 mg of extract in 25 ml of sea water media. The negative control used was seawater without extract. The sample was made in different concentration, i.e., 1000, 500, 100, 50, 25, and 12.5 ppm. Each vial contained 10 Artemia larvae, extract, and sea water up to 10 ml. Toxicity is measured by calculating LC_{50} after 24 hours observation. The experiment was repeated three times. The results were then compared with the negative controls. Further data obtained were calculated by Probit analysis using Minitab 17 program to determine LC_{50} with 95% confidence level.

Results and Discussion

The plant determination result showed that the plant examined is *Artocarpus altilis*. The cold extraction method was selected since it is unknown whether the secondary metabolites in this plant were heat resistant. Ethanol was selected as solvent since it is a universal solvent which is expected to dissolve the non-polar, semi polar and polar content of the fermented leaves. Phytochemical screening showed that both aerobically and anaerobically fermented leaves contained similar substances, *i.e.,* flavonoid, phenol, triterpenoid, steroid,

Table 4. Results of Probit Analysis

Extract	LC_{50} (ppm)
Aerob	712
Anaerob	1.927

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quinon, and saponin, except alkaloids that is only available in aerobically fermented leaves.

Determination of ash content is performed to evaluate mineral content. The ash contents were 13.5% and 20.5% for aerobically and anaerobically fermented leaves, respectively. Water soluble content test was performed to investigate polar compound in the extract. The water soluble contents were 18% and 23% for aerobically and anaerobically fermented leaves, respectively. This suggest that anaerob fermentation produce higher quantity of polar compounds.

Artemia salina was used in BSL test. It has thin and sensitive skin and are widely used in toxicity test. Foreign substances or compounds present in the environment will be absorbed into its body by diffusion. The *Artemia salina* larvae would die if the foreign substances or compounds are toxic. The purpose of this test was to determine the toxicicity level of widely used medicinal plant.

Extract with $LC_{50} \leq 30$ ppm is classified as very toxic category, whereas if $LC_{50} \leq 1000$ ppm is classified as toxic. If $LC_{50} \geq 1000$ ppm, it is classified as non-toxic.[8-10] The results of the study showed that LC_{50} of aerobic extract was 712 ppm, thus it can be categorized as toxic, while that of anaerobic extract is 1.927 ppm, or non-toxic to the Artemia larvae. This diffences might be caused by different composition of secondary metabollites, *i.e.,* alkaloid which might have side effect on certain living organism, such as *Artemia salina.*[10-12] Further research needs to be conducted to further confirm toxicity potency of *Artocarpus altilis* and determine the active compounds for development of anticancer agents.

Conclusions

Aerobically fermented *Artocarpus altilis* leaves had toxicity potency, while anaerobically fermented *Artocarpus altilis* leaves had no toxicity potency towards *Artemia salina* in BSL test.

Conflict of Interest

None declared.

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