Aloe vera polyphenols against fish stress through blood glucose

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Abstract— This study aims to analyze the blood glucose of koi fish exposed to polyphenol fraction of *A. vera*. It firstly found LC_{50} at the treatment of 150 mg.kg^{-1} that caused 50% mortality, so that the doses of *A. vera* fraction injected into the fish muscle as follows: Control (-) no treatment, Control (+) was given tannin/synthetic polyphenolic compound as much as 3 mg.kg^{-1} of body weight, treatment A= 75 mg.kg^{-1} of body weight, B= 100mg.kg^{-1} of body weight, C= 125 mg.kg^{-1} of body weight, respectively. After 72 h of injection, the blood plasm of Koi fish was taken to observe the stress effect on the blood glucose. Results showed that for blood chemistry, glucose with increased polyphenol fraction application due to higher stress level, with increased polyphenol dose. The clinical symptom after injected with polyphenol fraction of *A. vera* resulted in abnormal swimming, red spots, paleness, and hemorhage.

Keywords—polyphenol, fraction, *Aloe vera*, blood glucose, koi fish.

I. Introduction

Farming opportunity in Indonesia is still good enough to gain developing market potency. High transaction and trade of koi fish in Indonesia make the government, through the Ministry of Marine Affairs and Fisheries (MMAF), develop the national ornamental fish potency expected to be able to increase the local koi quality that is able to compete with imported koi either in domestic or international markets [1]. However, this fish culture still faces various problems to obtain good quality seed. The problems often appear are virus or bacteria-related diseases and toxic environmental factors [2], [3].

Natural processes, such as plant material decomposition, more or less contributes to phenol accumulation in aquatic environment [4], [5]. 96% phenolic residues came from industrial wastes, pharmacy and others, while the rest 4% from natural decomposition of plant, such as leaf, stem and root [6], [7]. For instance, *Aloe vera* that the inner leaf is taken as gel or latex [8], [9] is one of the plants containing polyphenol and its compound is a liquid pollutant in the fish body influences the metabolism [10], [11], survivorship, growth, and at the optimal dose, it functions as food, cosmetics [8], [12], protection against carcinogen-induced genetic damages or cancer [13], [14], [15].

The phenolic compound of *Aloe vera* is immune and antibacterial to bacteria *Aeromanas salmonicida* as well [16], [17]. In high dose, it can disturb the endocrinal system and high potential to disturb the body immune system and increase the fish vulnerability to secondary infection [18], [19]. The compound is found in *Aloe vera* and fish tissue [20] and chronically toxic and immunotoxic [21], [22].

II. Material and Method

Aloe vera powder production

leaf fronds of *Aloe vera* were sorted to obtain uniform leaf fronds in terms of color, size, and no leaf part damage. There were washed in running water to remove attached dirt and the leaf surface rubbed with clean cloth. The clean leaves were then cut in small pieces, weighed, and blended with addition of maltodextrin. The *Aloe vera* leaf gel-containing bucket was dried up to the gel changing into powder form.

The powder processing requires filler to accelerate the drying, prevent damage from heat, layer the flavor component,

increase total solid, and enlarge volume [23]. Filler used in powder processing in this study was maltodextrin. According to [24], maltodextrin is unsweet sugar, white flour-shaped, and soluble in the water, cheap, and can protect the capsule from oxidation, increase rendement, be easily resoluble, has relatively low viscosity.

Aloe vera fraction

Fractionation employed column chromatography method to separate the active compound of the extract into several fractions [25]. This method used stationary phase and mobile phase, in which the former was as silica *gel* G_{60} and the latter as eluent of the thin-layer chromatography of chloroform and methanol ratio (12:2). Various colors appeared as extract fractions of *A. vera* and held in the vial with color of silica gel G_{60}. After several different colors from the fraction of column chromatography product had been obtained, the fractions were evaporated using nitrogen, and the crust was taken and the UV-Vis testing was carried out.

The identification of the polyphenolic group of *A. vera* used UV-Vis spectrophotometer. As much as 3 ml of column chromatographic product sample was inserted into the cuvet. The spectrophotometer was scanned at the wavelength of 200 nm to 550 nm and calibrated using acetone. The amount of polyphenol (µg/gr) was assessed using the equation of $E^{1\%}_{1cm}$. The UV-Vis spectrophotometer analysis functions to indicate the presence or absence of conjugated double bond and can also determine the type of nucleus in the secondary metabolite compounds [26].

LC_{50} test

LC_{50} test is intended to find 50% mortality in 96 hours and to know the threshold of phenolic compound application using Range Finding Test (RFT) method. In acute exposure, organisms have contact with chemicals as single exposure or several exposures in short time, generally in number of hours or days [27], that can quickly be absorbed to give the impact [28]. The doses of polyphenol used in LC_{50} test for injection were 0 mg kg^{-1}, 50 mg kg^{-1}, 100 mg kg^{-1}, 150 mg kg^{-1}, and 200 mg kg^{-1}.

LC_{50} occurred in *Aloe vera* polyphenol dose of 150 mg/kg at 96 h, so that the dose of 125 mg/kg was assumed to be safer to use for toxicity test. According to [29], LC_{50} determination was based on LC_{50} polyphenol testing at 96 hours, while the synthetic phenolic compound, tannin, was administered at the dose of 0 mg kg^{-1}, 1 mg kg^{-1}, 2 mg kg^{-1}, 3 mg kg^{-1}, 4 mg kg^{-1}, and 5 mg kg^{-1} as positive control. LC_{50} was found at the dose of 4 mg kg^{-1}, so that the synthetic phenols used was 3 mg kg^{-1}.

Experimental design

The study utilized Complete Randomized Design. Treatments were different polyphenol extract of *A. vera*, positive control, and negative control, with 3 replications. Treatment A was polyphenol extract of *A. vera* of 75 mg/kg BW, Treatment B was polyphenol extract of *A. vera* of 100 mg/kg BW, Treatment B was polyphenol extract of *A. vera* of 125 mg/kg BW, Control (-) was without phenolic compound, and Control (+) = synthetic phenolic compound.

Application of *Aloe vera* fraction

Fifty percent of koi population was selected at uniform size, 15-20 g, and acclimated for 7 days of culture. At day-8, koi fish were injected with polyphenolic compound of *A. vera* at the dose of 75 mg/kg BW, 100 mg/kg BW, and 125 mg/kg BW, 3 mg/kg BW of synthetic polyphenol as positive control, and no treatment as negative control. After 72 hours, blood biochemical parameters of the fish were observed, while the clinical symptoms were recorded up to 96 hours.

Blood biochemistry parameters

Blood sample of Koi fish injected with polyphenol of A. vera was taken after the fish were fasted for 24 h. According to [30], to avoid blood clotting at blood sampling or storage, the syringe and the blood tube was given 0.1 mL of 2.7% anti-coagulant EDTA. Blood sampling was done at the base of tail (between anal fin and lateral line) using 1 mL syringe with the needle directed to the lower part of the spine with a slope below 45oC. The collected blood was inserted into a blood tube and stored in the refrigerator. The blood plasm was taken as liquid of the blood stored in the refrigerator, then the blood parameters, such as glucose.

III. Result and Discussion

UV-Vis – *Aloe vera* fraction test

Polyphenol fraction through ultraviolet spectrophotometer-based compound characterization gave spectral data, absorbance, and maximum wavelength of the compound isolate compared with the standard polyphenol solution of tannin (Figure 1).

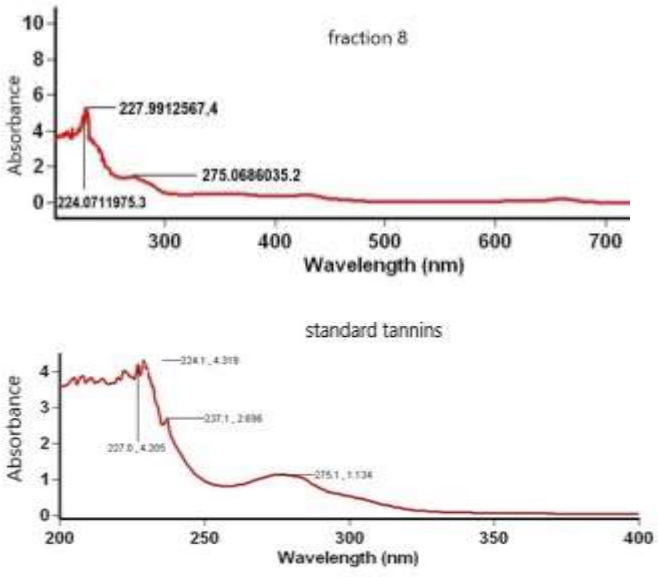

Figure 1. UV-Vis spectra of isolate compound of A. *vera* fraction 8 and standard polifenol (tannin).

Fraction absorbance qualitatively shows a strong peak at the wavelength of about 227.0 nm and weak peak at the wavelength near 275.0 nm (Table 1). According to [31], the

isolate with spectra of 210-280 nm comes from flavonoid group. It is supported by [32] that maximum absorbance range of the phenolic compound occurs at the wavelength of 200-400 nm. Based on the qualitative measurements using UV-Vis spectrophotometer, the fraction 8 of *Aloe vera* extract contains much phenolic compounds.

Table 1. Maximum wavelength of ultraviolet spectroscopy of *Aloe vera* and standard polyphenol (tannin)

Sample	Wavelenght λ_{maks} (nm)	Absorbance A_{maks}
Aloe vera	λ1 = 224.99	A1 = 1.561
	λ2 = 227.07	A2 = 0.824
	λ3 = 275.82	A3 = 0,.473
Polifenol standard	λ1 = 224.03	A1 = 1.134
(Tannin)	λ2 = 227.05	A2 = 2.694
	λ3 = 275.05	A3 = 1.377

Blood glucose

Observations during the study on the effect of *A. vera* polyphenolic fraction on the change in blood glucose level showed different effects between treatments. Mean blood glucose level (mg dL^{-1}) of koi is presented in Figure 2.

The highest blood glucose of koi fish (Figure 2) was recorded in positive control (K+) at the synthetic polyphenolic compound administration, 3 mg tannin kg^{-1} BW with glucose level of 146.87mg dL^{-1} and the lowest at the negative control (K -) of 66.67mg dL^{-1} and *A. vera* polyphenol treatment of 75 mg kg^{-1} BW with mean glucose level of 83.33 mg dL^{-1}. Treatment B (100 mg kg^{-1} BW) yielded mean glucose of 103 mg dL^{-1} and treatment C gave mean glucose of 132.33 mg dL^{-1}. According to [33], blood glucose of gourame ranges from 60 mg dL^{-1} – 90 mg dL^{-1} in normal condition. while that of silver bards ranges from 40 to 90 mg dL^{-1} [34]. It is in agreement with negative control and treatment A.

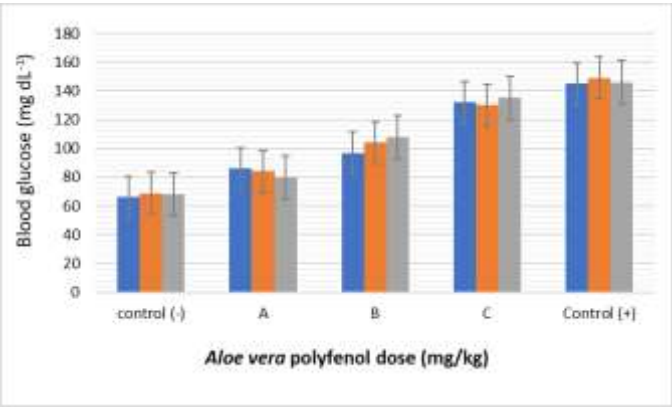

Figure 2. Relationship between *Aloe vera* polyphenol dose and Koi fish glucose (mg/dL)

Glucose level rose when administered with polyphenol. According to [35] and [36], polyphenol injected into the muscle of koi fish is responded as stressor indicated with increased blood glucose. In certain limit, every organism has sustainability or tolerance level to environmental changes. When the fish are stressed, they will show primary response as increased stress hormone level, such as cortisol and catecholamine of internal cell and secondary response as increased glucose level [37]. In stress condition, according to [38], increased glucocorticoid occurs and makes the blood glucose level rise to overcome high energy requirement.

The energy need from glucose for stress handling could be met if the blood glucose could immediately enter the cell and then metabolized to fulfil the physiological need of the body and energy need. High glucose supply will stimulate glycogenesis and lypogenesis [39], [40]. The success of glucose supply into the cell is determined by insulin performance. However, during the stress, the insulin is inactivated so that the glucose utilization of the cell is closed [41], [42].

In stress condition, receptor organ will receive information to be sent to the hypothalamus, then chromaffin cell will secrete catecholamine. This hormone will suppress the secretion of insulin hormone that functions to help supply glucose into the cell, so that glucose level in the blood rises. It will also activate enzymes involved in the catabolism of liver and muscle glycogen storage. Phenol exposure makes the blood glucose level rise due to increase in body metabolism, blood glucose homeostasis and disturbed hormone [43].

Clinical symptoms

Observations on the clinical symptoms of koi seed after injected with polyphenol show conditions as described in Table 2 in which polyphenol is the chemical that can make the fish be unstable, internal organ damage, pale, and red patches on the operculum causing the number of erythrocytes, hematocrites and haemoglobin fall down. Polyphenol toxin is a cytotoxic bioactive compound. This toxin can disturb or damage the cell membrane which can interrupt the compound transport in and out the cell and causes rupture of the blood vessels so that wounds occur, and hematopoietic organs cannot compensate for the loss of erythrocytes from bleeding [43], [44].

Table 2. Pathological changes in koi seed after injected with phenolic fraction of A. *vera*

After injection	Clinical symptoms	Internal organ observation
24 h	• Several fish show abnormal swimming on the surface, and low appetite. Fish gill and skin are still normal.	• No dead fish.
46 h	• Abnormal swimming on the surface or staying on the tank basin. • There are several red patches on the operculum in pale color. • Highly slimy and	• No dead fish.

	color of body part seems to be paleness.	
72 h	• Abnormal swimming near the surface and staying on tank basin. • Operculum is pale • Highly slimy and some body part starts turning to blackish and pale.	• Dead fish with normal organ, but there is water inside the abdominal cavity and intestine.
96 h	• Abnormal swimming on the surface, slant movement. • Swelling of the abdomen.	• Dead fish with damaged internal organ. • Haemorhage on the liver. • Eyes come out.

IV. Conclusion

The study shows polyphenol application of *A. vera* extract at the dose of 75 mg kg^{-1}, 100 mg kg^{-1}, and 125 mg kg^{-1} caused stress in fish. The stress condition could be seen from blood biochemistry, in which higher blood glucose and cortisol from polyphenol fraction administration indicates higher stress condition, and higher dose administration gave lower ALP enzyme. The clinical symptom after injected with polyphenol fraction made the fish swim abnormally near the surface and then stay on the pond bottom. The operculum was pale, highly slimy, and the body seemed to turn blackish. Therefore, the use of polyphenol compound needs to be considered, since it could give bad impact on the normal fish. The use of 75 mg kg^{-1} gave similar biochemical effect to the negative control and caused the lightest stress approaching to normal condition.

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