

# 2\_Hypoglycemic effect of instant aloe vera\_Riyanto.pdf

Hypoglycemic effect of instant *aloe vera* on the diabetic rats<sup>1</sup>Riyanto and <sup>2\*</sup>Wariyah, Ch.<sup>1</sup>Department of Agrotechnology, Faculty of Agroindustry, Mercu Buana University of Yogyakarta, Jl. Wates Km 10 Yogyakarta 55753, Indonesia<sup>2</sup>Department of Food Technology, Faculty of Agroindustry, Mercu Buana University of Yogyakarta, Jl. Wates Km 10 Yogyakarta 55753, Indonesia

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## Abstract

Instant *aloe vera* contains phenolic compounds which has antioxidative activity. However, this product is hygroscopic and damaged easily during storage. The critical condition of the instant occurs at the moisture content of  $12.52 \pm 0.24\%$  (wb). Increasing the moisture content could accelerate oxidation of the phenolic compounds, thus decrease the antioxidative activity. Previous research showed that the antioxidative activity of instant *aloe vera* could lower the blood glucose. The purpose of this study was to evaluate the hypoglycemic activity of instant *aloe vera* during storage until the critical condition. The hypoglycemic effect was determined with the *in vivo* method using diabetic Wistar rats as experimental animals. The diabetic rats fed with a standard feed combined with instant *aloe vera* which has been stored at various storage time i.e. 0, 2, 4, 6, 8 weeks and used normal rats fed without instant *aloe vera* as a control. The blood glucose was analyzed every week until 4 weeks. The research showed that the diabetic rats fed with standard feed without instant *aloe vera* had high blood glucose (219.40 mg/dL) after 4 weeks treatment. Otherwise, the blood glucose of diabetic rats fed with instant *aloe vera* decreased from 214.00 mg/dL to 97.57 mg/dL after 4 weeks.

## 1. Introduction

Instant *aloe vera* was made from *aloe vera* leaves and was processed by microencapsulation using spray dryer and maltodextrin as an encapsulating agent. Instant *aloe vera* contains phenolic compound of  $1.64 \pm 0.09 \mu\text{g/g}$  dry matter (Wariyah and Riyanto, 2015). According to Sultana and Anwar (2008), the phenolic compounds in *aloe vera* gel are flavonoid i.e kaempferol, quercetin, and myricetin of about 257.70; 94.80 and 1283.50 mg/kg, respectively. Flavonoids have an antioxidative activity indicated by their ability to capture free radicals of DPPH (*1,1-Diphenyl-2-picrylhydrazyl*) (Hu *et al.*, 2005). Joseph and Raj (2010) stated that the bioactive substances in *aloe vera* can lower blood glucose. Jasmine and Daisy (2007) found that methanol soluble extract of *Eugenia jambolana*, which also contains flavonoids, could lower blood glucose, so the hypoglycemic effect is estimated to be related to the flavonoids. Yagi *et al.* (2009) proved that consumption of *aloe vera* fraction about 10 ppm over a period of 6

weeks, could lower the blood glucose of the experimental animals.

The processing of instant *aloe vera* used *aloe vera* powder (dried *aloe vera*) as a raw material and was done in consecutive stages i.e. reconstituting *aloe vera* powder using aquadest at a ratio of 1 : 120 (w/v), filtering the solution, and then maltodextrin added as an encapsulating agent. The solution was fed into a spray dryer. Wariyah and Riyanto (2015) showed that instant *aloe vera* had high antioxidative activity and the ability to capture free radicals with a percentage of RSA (*Radical Scavenging Activity*) was about  $16.34 \pm 1.14\%$  (fresh instant) and  $2.34 \pm 0.37\%$  (instant at critical condition), and the inhibition of lipid peroxidation was  $39.34 \pm 1.58\%$  (fresh instant) and  $21.34 \pm 0.16\%$  (instant at critical condition). The antioxidative activity of instant *aloe vera* decreased during storage caused by contact with light, heat, and oxygen, which accelerated oxidation reaction of flavonoid (Özkan and Bilek, 2014).

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Decreasing of the antioxidative activity of instant *aloe vera* could affect the hypoglycemic activity because it relates to the free radical neutralization by antioxidant. According to Barlett and Eperjesi (2008), hyperglycemia and diabetes cause an increase in free radical or ROS (Reactive Oxygen Species) i.e. superoxide, hydrogen peroxide and singlet oxygen. ROS is capable of damaging lipid membranes, proteins, nucleic acids, and carbohydrates via oxidation, resulting in the formation of cytotoxic chain reactions. Therefore, an antioxidant may neutralize free radicals effectively is needed. The purpose of the study was to evaluate the hypoglycemic activity of instant *aloe vera* during storage until reached critical condition.

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## 2. Materials and methods

### 2.1 Materials

*Aloe vera* leaves (*Aloe vera* var. *chinensis*) with harvesting age of 1.5 – 2.0 years were obtained from the Loano District in the Purworejo Regency, Central Java Province, Indonesia. Maltodextrin DE 20 as an encapsulating agent was purchased from Brataco Chemika and sodium chloride for adjusting relative humidity during storage of the instant from Merck. Plastic film for packaging of *aloe vera* instant used the High Density Polyethylene (HDPE) with 0.80 mm thickness was obtained from "40" store at Yogyakarta, Indonesia.

### 2.2 Methods

*Aloe vera* leaves were processed into powder before microencapsulated into instant and the processing of powder referred to Wariyah and Riyanto (2011). Microencapsulation of *aloe vera* powder into the instant referred to Wariyah and Riyanto (2016) and prepared as follows: *aloe vera* powder was reconstituted by using distilled water at ratio of 1/120 (w/v) and then mixed with 7.5% (w/v) maltodextrin with constant stirring using a magnetic stirrer (Stir plate Nuova II) at 700 rpm for 45 minutes. The solution was fed into the spray dryer (Lab Plan SD-05) at an inlet temperature of 130°C and an outlet temperature of 103°C, an air flow rate of 50m<sup>3</sup>/h, and a solution flow rate of 350 mL/h. The powders (instants) obtained were packaged in plastic film with 0.80 thickness and stored at various storage time of : 0, 2, 4, 6, 8 weeks, at a relative humidity of 75% (adjusted with N<sub>2</sub>) and temperature of 25°C. The hypoglycemic effect was determined by the *in vivo* method (Kabir *et al.*, 1998) using Wistar rats with an age of 3 – 4 months and a weight of between 240 – 260 g. The rats were

obtained from the *Integrated Research Centre Labs.*, Gadjah Mada University of Yogyakarta, Indonesia.

### 2.3 Determination of instant *aloe vera* intake for rats

Each of sample instant *aloe vera* with different storage time was used as animal feed combined with standard feed (Reeves *et al.*, 1993). The diabetics rats were prepared by alloxan induction during 5 days as much as 125 mg/kg body weight. Normal rats and diabetic rats fed with standard feed without instant *aloe vera* were used as a control. The animals for each treatment (n=6) were observed for 4 weeks. The amount of instant added to the standard feed was determined by preliminary research based on their reducing power (Yen and Duh, 1994) compared with commercial vitamin E as a standard. The vitamin E contains  $\alpha$ -tocopherol equivalent to 100 IU per capsule. Intake of instant *aloe vera* for rats was equalized with the adequate intake of antioxidant to prevent degenerative disorder up to 400 IU/day/adult. Moreover, the value was multiplied by a conversion factor (0.018) to fit the animal feed. The hypoglycemic effect was calculated by the change in blood glucose of diabetic rats before and after being fed with the sample. The rats were fed with the treatment for a period of 4 weeks and blood glucose was analyzed each week using the GOD-PAP method (Goni *et al.*, 1996).

### 2.4 Design of experiments

This study used completely randomized design with the storage time of instant *aloe vera* a factor. The differences among the treatments were determined by F test, and the significant difference between samples was examined by Duncan's Multiples Range Test (DMRT) (Gacula and Singh, 1984).

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## 3. Results and discussion

### 3.1 Phenolic content and antioxidative activity of instant *aloe vera*

Table 1. Characteristics of instant *aloe vera*

Characteristics	Instant <i>aloe vera</i>
RSA (Reactive Scavenging Activity) (%)	15.32±1.14
Inhibition of lipid peroxidation (%)	36.87±1.58
Moisture (%wb)	5.65±0.62
Total phenol ( $\mu$ g/g dry matter) <sup>a</sup>	1.64±0.09

<sup>a</sup>Wariyah and Riyanto, 2015.

Table 1 shows the characteristics of instant *aloe*

*vera*, which related to hypoglycemic effect. The phenolic content of instant *aloe vera* was  $1.64 \pm 0.09$   $\mu\text{g/g}$  dry matter and the antioxidative activities which indicated by their RSA (*Reactive Scavenging Activity*) and the inhibition of lipid peroxidation were  $15.32 \pm 1.14\%$  and  $36.87 \pm 1.58\%$ , respectively. Instant *aloe vera* had a high antioxidant activity, and according to Sultana and Anwar (2008), the antioxidative properties shown by its ability to capture DPPH free radicals. The antioxidative activity could decrease during storage due to contact with heat, oxygen, and light which causes the oxidation of flavonoids (Nawar, 1985). Wariyah and Riyanto (2015) stated that RSA value of instant *aloe vera* significantly decreased at 5 weeks storage time, while the inhibition of lipid peroxidation decreased after 9 weeks. Therefore, important to study the effect of storage time on the hypoglycemic activity of instant *aloe vera*.

### 3.2 Reducing power of instant *aloe vera*

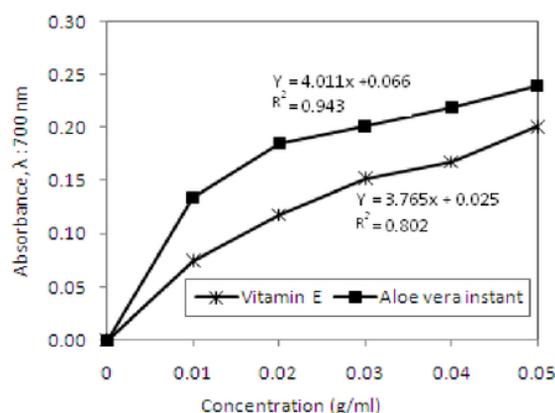


Figure 1. Reducing power of vitamin E and instant *aloe vera*.

Reducing the power of instant *aloe vera* and vitamin E was compared to determine the rat's intake. Consumption necessary of antioxidant for preventing degenerative disorder is about 400 IU per day, whereas a 0.40 g capsule of commercial vitamin E containing 100 IU  $\alpha$ -tocopherol. Therefore, the instant *aloe vera* given to the rats were equalized with 400 IU  $\alpha$ -tocopherol in vitamin E based on their reducing power. The relative reducing power stated by absorbance value is shown in Figure 1. The higher the absorbance, the greater its reducing power or the higher its antioxidative activity as shown by the regression equation of the two samples.

Figure 1 shows the relationship between absorbance and sample weight (instant and vitamin E). The regression equation of vitamin E is :

$$y = 4.011x \pm 0.066 \quad (1)$$

If the x parameter is equal to 1.6 g commercial vitamin E (equivalent with 400 IU  $\alpha$ -tocopherol), so the y parameter  $y = 6.4836$ . The instant *aloe vera* regression equation is:

$$y = 3.765x \pm 0.025 \quad (2)$$

To satisfy  $y = 6.4836$ , the instant weight (x) = 1.70 g. It means that to provide the adequate daily intake of antioxidant about 400 IU/day/adult is needed about 1.70 g instant *aloe vera* for a human with a 70 kg body weight or 0.03 g for a rat with a 200 g body weight.

### 3.3 Rats body weight and hypoglycemic activity of instant *aloe vera*

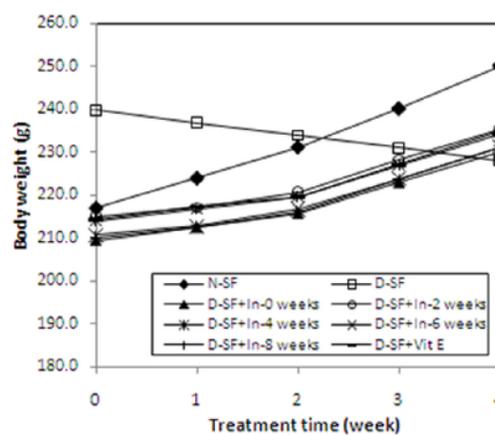


Figure 2. Profile of rat body weight during treatment.

Figure 2 shows the profile of rats body weight during 4 weeks treatment, and Figure 3 shows the rats blood glucose profile with treatment of normal rats fed with standard feed (N-SF), diabetic rats fed with standard feed (D-SF), and diabetic rats fed with standard feed combined with instant *aloe vera* (D-SF±In) at various storage time (0.0 to 8.0 weeks). Hypoglycemic activity expressed as the ability of the sample to decrease blood glucose level. The body weight of normal rats fed with standard feed increased during the 4 weeks treatment, while the body weight of diabetic rats with standard feed decreased. Kuzuya *et al.* (2002) and Al Tera (2011) described that decreasing body weight is one symptom of diabetes mellitus. Diabetic patients undergo weight loss when blood glucose can not be absorbed into the cells and the energy requirement is taken from body fat. Akbarzadeh *et al.* (2007) found that diabetic rats induced with streptozotocin the body weight decrease in comparison with a normal rat. This study resulted that the body weight of diabetic rats fed with instant *aloe vera* (D-SF±In- 0 to 8 weeks) increased with a similar profile in comparison with N-SF. The longer the storage time, the lower increasing of the body weight.

Figure 3 shows the blood glucose profile of diabetic rats fed with or without instant *aloe vera*. The diabetic rats fed without an instant *aloe vera* showed high stable blood glucose (>200 mg/dl) during the 4 weeks of treatment. Whereas, normal fasting blood glucose was <110mg/dl and 140 mg/dl after meals (Kuzuya *et al.*, 2002). The blood glucose of diabetic rats fed with instant *aloe vera* decreased to normal levels by the fourth week of treatment. Winarsi *et al.* (2014) showed that the diabetic rats given the Ethanolic Cardamon Leaves Extracts (ECL) which contain flavonoid for 7 consecutive days, decreased in their blood glucose level. Jasmine and Daisy (2007) also stated that the blood glucose of diabetic rats fed with flavonoid extract from *Eugenia jambolana* for 30 days decreased from 534.60 mg/dl to 206.80 mg/dl.

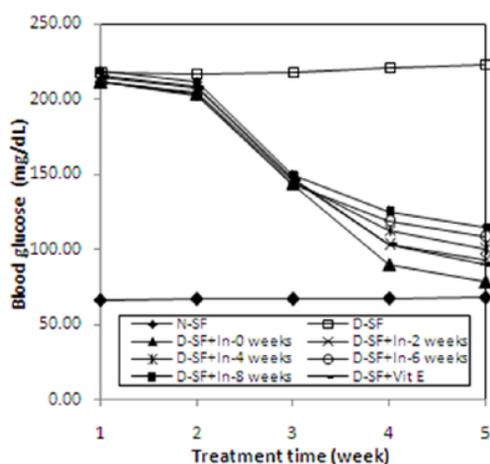


Figure 3. Profile of blood glucose during 4 weeks treatment.

Moreover, flavonoids are capable of stimulating insulin secretion from the pancreas and of excreting an insulin secretion inhibitor. According to Barlett and Eperjesi (2008), hyperglycemia can increase oxidative stress causing an increase of ROS such as superoxide, hydrogen peroxide and singlet oxygen. ROS is capable of damaging lipid membranes, proteins, nucleic acids, and carbohydrates through oxidation, which can result in the formation of cytotoxic chain reactions so that the glucose metabolism is impaired. Increasing of ROS formation leads to more NADH and FADH<sub>2</sub> enter to electron transport chain (Suarsana *et al.*, 2011 as cited in Winarsi *et al.*, 2014). The increase of electron transport rate leads to the contribution of free radicals formation and cause a more severe diabetic. Therefore, neutralizing free radicals by use of antioxidants such as the flavonoids found in *aloe vera* is an important step in decreasing the prevalence of diabetes mellitus.

#### 4. Conclusion

Instant *aloe vera* resulted from microencapsulation of *aloe vera* powder has hypoglycemic activity. The activity can provide the needed antioxidant to prevent degenerative disorder, especially diabetes mellitus. The hypoglycemic activity of instant *aloe vera* was affected by the storage time, the longer the storage time, the lower its hypoglycemic activity. The instant *aloe vera* which packaged in polyethylene plastic with 0.80 mm thickness until eight weeks and consumed about 0.03 g for rats or 1.70 g for human/day could lower blood glucose to the normal level.

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