

## Effect of AVG on Anthocyanin and Antioxidant Activity of Bignay Fruit Juice

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**Abstract**— Consumption of fruit juices product containing various bioactive compounds that are good for health is a current trend. Bignay fruit is a tropical fruit that contains high anthocyanin and can increase its antioxidant ability by adding aloe vera gel (AVG). Bignay fruit also contains organic acids, namely gallic, ferulic, and ellagic acids. AVG is associated with many transparent or colorless polysaccharides and contains functional bioactive chemical compounds. This research aimed to evaluate the effect of AVG concentration on the anthocyanin and antioxidant activity of bignay fruit juice. This research design used a completely randomized design of one factor, namely the concentration of AVG. The formulation of bignay fruit juice with the addition of AVG at concentrations of 25%, 50%, and 75% at a temperature of (6±1) °C was carried out. The observed variables included total anthocyanins, total flavonoids, vitamin C, antioxidant activity, viscosity, and acidity. All experimental units were repeated three times. Periodic observations of bignay fruit juice were carried out on storage 0, 3, 6, 9, 12, and 15 days. The AVG concentration significantly affected the total anthocyanin, antioxidant activity, and viscosity of bignay fruit juice. The best formula for bignay fruit juice is the addition of AVG with a concentration of 25%. The bignay fruit juice notes a large number of functional compounds until the ninth day of storage. Bignay fruit juice can be a recommendation for health-conscious consumers to fulfill their functional drink desires.

**Keywords**— Aloe vera; antioxidant activity; anthocyanin; bignay fruit; juice.

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### I. INTRODUCTION

The interest in fruit juice containing bioactive compounds has increased in current years. The change in consumption to accommodate juice containing various bioactive compounds has resulted in new products. Bignay (*Antidesma bunius*) is a fruit that has the potential to be developed into juice products. The bignay fruit's red to purplish (violet) color is known as procyanidin B1 and procyanidin B2 anthocyanins. Bignay fruit also contains phenolic organic acids, namely gallic, ferulic, and ellagic. The edible part of the bignay fruit is 65-80% of the total weight of the fruit. Bignay fruit contains 90-95 g of water, 6.3 g of carbohydrates, 0.8 g of fat, 0.7 g of protein, 3.2-120 mg of calcium, 22-40 mg of phosphorus, 0.1-0.7 mg of iron, 8 mg of vitamin C and 10 IU of vitamin A and contains the energy of 134 Kj/100g per 100 g of weight. The most abundant organic acid in bignay fruit is ascorbic acid [1].

The medicinal properties of bignay fruit mentioned in previous research might also additionally enlarge the usage of

ripe fruit as a medicinal product[2]. Extracts and phytoconstituents isolated from bignay fruit have been shown to produce various pharmacological responses, including cytotoxic, antiradical, antidysentery, anticancer, and blood circulation improvement[3]. Some studies have mentioned bignay fruit show off antibacterial, an inhibitor of glucosidase, antidiabetic, and important antioxidant additionally[3]. Bignay fruit has a bitter and sweet taste[4]. Anthocyanins found in the flesh of the bignay fruit are natural dyes as an alternative to synthetic dyes that are very safe and do not negatively impact health. To maximize bignay's bioactive compound and antioxidant activity, fruit juice can be added with AVG.

AVG is associated with many transparent or colorless polysaccharides obtained from the mucilage of Aloe vera leaf (*Aloe barbadense*. Miller) [5]. According to [6], AVG contains more than 200 functional bioactive chemical compounds. The biological activity of AVG is basically because of the synergy of numerous compounds [7]. AVG

covers polysaccharides, proteins, lipids, minerals, and phenolic compounds [8]. AVG has antioxidant abilities [9], which may increase food's stability and nutritional value [10]. Health advantages of AVG include decreasing low-density lipoprotein, growing high-density lipoprotein and blood sugar in diabetics, and enhancing the immune system [11]. However, AVG has a sour flavor and brings about unsightly flavor sensations [12]. Aloe juices with certain blends are very popular, for example, lemon juice, the soluble fiber in a diet beverage, vitamin B, and vegetable juices in a healthy beverage. Adding some fruit juices is an easy technique to reduce the bitterness of the aloe beverage [13]. However, the study of appropriate formulation of AVG blended with some fruit juices is still lacking.

The formulation of bignay fruit blended AVG into a beneficial juice that provides health effects is a product diversification step. The content of anthocyanins, flavonoids, antioxidant activity, and viscosities is a composition that is expected to be the advantage of bignay fruit Juice. This technique has not existed in preceding research and wishes to be achieved in intensity research. The weakness of this product is the deposition due to the high total flavonoid content in bignay fruit [14]. The stability of the juice made from bignay fruit and AVG is an important aspect that needs attention to increase its shelf life. Anthocyanin levels, flavonoids, antioxidant activity, vitamin C, thickness, and acidity are important components of bignay fruit juice functional drinks. This study aims to evaluate the effect of AVG concentration on the anthocyanin and antioxidant activities of bignay fruit juice.

## II. MATERIALS AND METHOD

This research used a completely randomized design with one factor: the concentration of AVG is 25%, 50%, and 75%, stored at temperatures ( $6 \pm 1$ ) °C. The observation periodically of 0, 3, 6, 9, 12, and 15 days. Each treatment was replicated three times. The main ingredients used are optimally ripe bignay fruit 125 days from flowering, *Antidesma bunius* L. Another ingredient is the leaves of Aloe barbadense Miller whose plant is 1 year old. There are no white spots on the skin of aloe vera leaves. The materials used in the analysis are NaCl, distilled water, diluent solution, agar medium, NaOH. This research was carried out at the Processing Laboratory of Warmadewa University. The equipment used is digital scales, blender, basin, knife, tablespoon, mixing spoon, filter, plastic cup, plastic bottle, cutting board, chiller, beaker, measuring cup, dropper, analytical balance, petri dish, Erlenmeyer, funnels, test tubes, test tube racks, pH-meters, viscometers, and incubators.

Analysis of anthocyanin content was carried out using the pH differential method. A certain volume of bignay fruit juice is dissolved in two buffer solutions. The first solution was dissolved in 0.025 M KCl buffer pH 1.0, and the other solution was dissolved in 0.4 M sodium acetate buffer pH 4.5. The number of samples used was adjusted so that the absorbance at vis-max gave an absorbance value in the spectrophotometer's linear range. Scanning wavelengths in the range of 200 nm – 750 nm were also carried out for sample solutions in both buffers to determine anthocyanin content and determine vis-max sample measurement. Next,

absorbance measurements were taken, and the results were calculated based on Equation 1.

$$A = (A_{\text{vis-max}} - A_{700\text{nm}}) \text{ pH}1.0 - (A_{\text{vis-max}} - A_{700\text{nm}}) \text{ pH}4.5 \quad (1)$$

The total flavonoid content and aqueous sample solutions of various concentrations were used to prepare a reaction mixture of 100 L of sample solution and 100 L of 2% (w/v) AlCl<sub>3</sub> solution to give a final sample concentration of 0–100 g/mL. The samples were incubated at room temperature for 30 minutes. Then the optical absorbance was determined at 437 nm using a spectrophotometer. A quercetin calibration curve is plotted using a similar method between the quercetin content (x-axis) and the corresponding absorbance (y-axis). Total flavonoid content was extrapolated and expressed as mg quercetin equivalent (mg QE equivalent/g extract). Vitamin C levels were determined by iodine titration. The sample was put into an Erlenmeyer with as much as 5 ml, 20 ml of distilled water, and a few drops of starch solution added. Then it was titrated with 0.01 N iodine solution until it turned blue. Each ml of Iodine solution is equivalent to 0.88 mg of ascorbate. The following formula can calculate vitamin C levels Iodometric method:

$$\text{Vit C (mg/100g)} = \frac{(V_{I_2} \times 0.88 \times F_p) \times 100}{W_s (\text{gram})} \quad (2)$$

Description:

- V<sub>I2</sub> = Iodine Volume (mL)
- 0.88 = 0.88 mg of ascorbic acid is equivalent to 1 mL of 0.01 N I<sub>2</sub> solution
- F<sub>p</sub> = Dilution Factor
- W<sub>s</sub> = Sample weight (grams)

Furthermore, its viscosity was evaluated using a UV-Vis spectrophotometer at 281 nm. The acidity value of the Aloe-bignay beverage was measured with a digital pH meter (Hanna HI 8424, Romania) by standardizing the pH meter before. Determination of antioxidant activity was then carried out with the DPPH method, as explained by Shah and Modi, 2015. The antioxidant activity percentage was calculated with the formula:

$$\text{The activity of antioxidant \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \quad (3)$$

### A. Bignay Extraction

Bignay fruit is separated from the bunch, and the washing process can be carried out optimally. Washing is done many times until the bignay fruit is free from dirt at harvest, then the fruit is drained. Bignay fruit has a bunch of different ripeness, so the sorting of bignay fruit is done to separate between immature fruit (green), ripe (red), and very ripe (purple) (Fig. 1). The fruit used in this beverage is ripe fruit with a red color. The ripe bignay fruit is then crushed with a blender. In this process, the bignay fruit is mixed with water to get the juice from the bignay fruit. Bignay fruit that has been crushed is then filtered. This process will produce pulp in the form of bignay seeds and bignay skin that cannot be destroyed. The fruit juice that has been separated from the pulp is then heated. The purpose of this heating is to kill the microbes present in the juice. Heating is done with high heat but for a short time. The heated bignay juice is allowed to cool and then filtered

again to separate the bignay juice from the bignay skins, which are also filtered with the bignay juice in the first filter.



Fig. 1 Bignay fruits with different ripeness

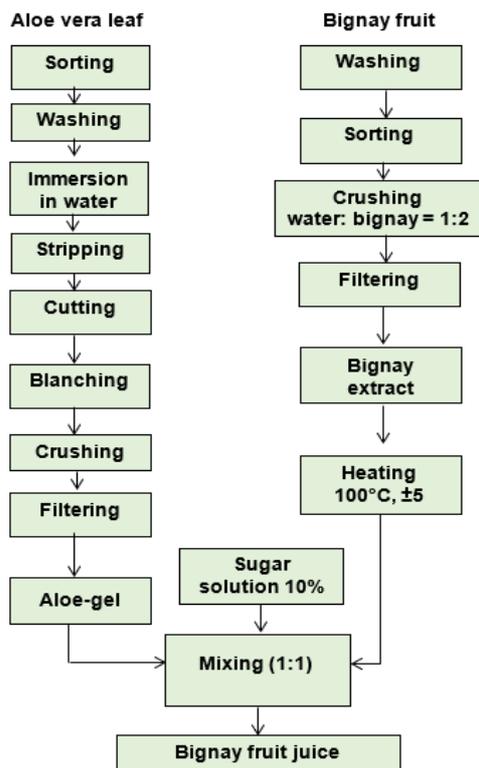


Fig. 2 Formulation of bignay fruit juice

### B. AVG extraction

The first step of extracting the gel from Aloe vera leaves is washing with water to remove dirt adhering to the leaves. The stripping of Aloe vera leaves was carried out  $\pm 2$ mm thick from the surface to get the gel. Cutting and removing the thorns on the leaves is done to facilitate the stripping and destruction process. AVG that has been cut and cleaned from

the skin is blanched at  $80^{\circ}\text{C}$  for 5 minutes to minimize yellow zap, which will affect the beverage's taste. The blanched gel was blended, filtered, and heated for 5 minutes for enzyme inactivation. The filtering was repeated to obtain a fibrous AVG.

### C. Formulation of Bignay Fruit Juice

The formulation consists of 3 proportions, namely: formula 1 (AVG 25%), formula 2 (AVG 50%), and formula 3 (AVG 75%). The additive used a sugar solution as a sweetener for a bignay fruit juice that the concentration is 20%. Beverages are packaged in 100 ml plastic bottles that have been sterilized. Once packaged, the bignay fruit juice is stored at  $6\pm 1^{\circ}\text{C}$ . The variables of total anthocyanins, total flavonoids, vitamin C, antioxidant activity, viscosity, and acidity were analyzed periodically on days 0, 3, 6, 9, 12, and 15. Formulation of bignay fruit juice as in Fig 2.

## III. RESULTS AND DISCUSSION

### A. Anthocyanins

The results showed that bignay fruit considerably affects the total anthocyanin. The result of the research is that the AVG concentration significantly affects the anthocyanin content of bignay fruit juice. A higher percentage of AVG fruit decreased the total anthocyanins of juice, namely 10.95-12.55% (Fig. 3). AVG does not contain anthocyanins; an increase in AVG concentration will reduce the absolute total anthocyanin in bignay fruit juice. Anthocyanins are included in the group of essential pigments in plants with a flavylum cation ( $\text{AH}^+$ ) structure acting as an acid. This structure was immediately attributed to its antioxidant activity.

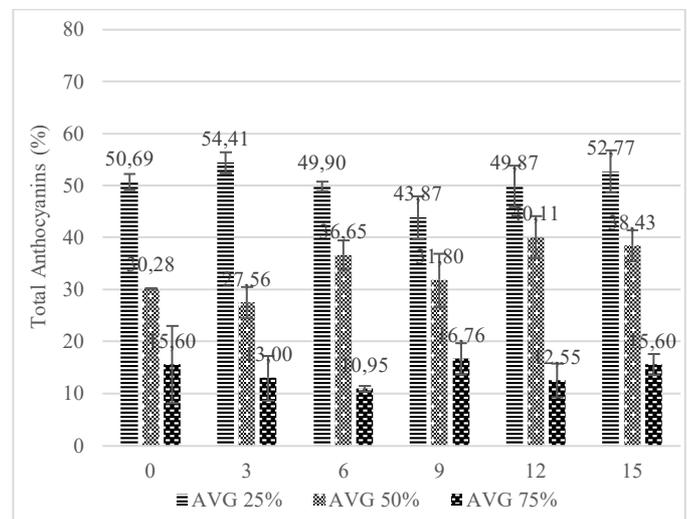


Fig. 3 Total anthocyanins of bignay fruit juice (%)

Most of anthocyanins' functional and sensory properties can be determined by their chemical reactivity[11]. The bignay fruit contains components that improve health and a supply of natural antioxidants[3]. In line with the opinion [15], bignay fruit juice contained 48,931 mg/100 ml antioxidants. At a temperature of  $6\pm 1^{\circ}\text{C}$ , total anthocyanins are stable for 15 days of storage. The bignay fruit extract has the potential as an antioxidant and antimutagenic, which does not show genotoxicity[16].

### B. Flavonoids

AVG presented a non-considerable result to the flavonoid content of bignay fruit juice. The average value of the total flavonoids of bignay fruit juice is 112.94- 302.5 mg/100g QE. The addition AVG shows the total flavonoids that tend to be stable, as seen in Fig. 4. While the addition of AVG showed the total flavonoids of aloe-bignay beverage decreased during storage 15 days. Total flavonoids are one of the essential quality indexes of Aloe-bignay beverage, and their miles are involved with overall antioxidant activity[17][18]. The total flavonoids in bignay fruit are higher than AVG. In a study conducted by[19] total flavonoid bignay 120.39 mg/100 g FW. The flavonoid content turned into 138.60-289.60 mg catechin (CE)/100 g DW [1]. The outcomes of the examination showed that AVG containing flavonoids of  $62.70 \pm 0.44$  mg EDTA eq./100ml. Determination of total flavonoids of Bignay fruit juice is of great importance to their qualities.

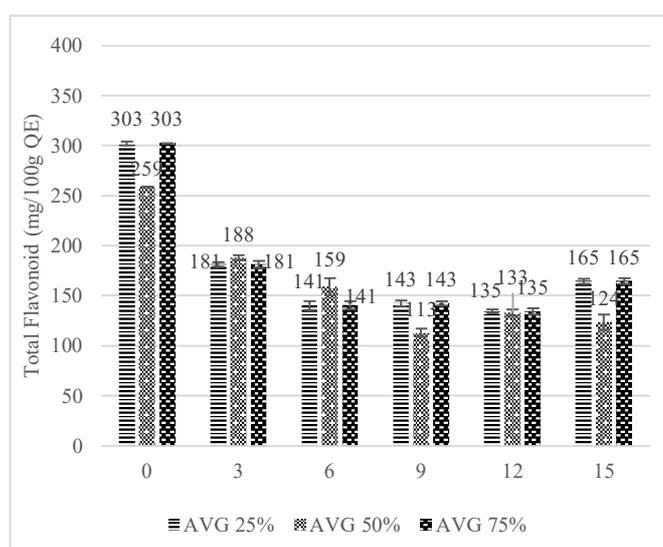


Fig. 4 Total flavonoids of bignay fruit juice (mg/100g QE)

### C. Vitamin C

The vitamin C in bignay fruit juice is equivalent to ascorbic acid. The results showed that the concentration of AVG was non-significant different from vitamin C of bignay fruit juice. Vitamin C of bignay fruit juice with AVG was 53.98-180.71 mg/100g (Table 1). The ascorbic acid of bignay fruit was 26.14 - 48.93 mg/100 ml [11]. A smaller concentration of AVG increases the vitamin C of the bignay fruit juice. Vitamin C in bignay fruit juice stored at  $6 \pm 1^\circ\text{C}$  indicates high relativity. The juice storage at these temperatures can maintain the vitamin C content. The vitamin C of bignay fruit juice was discovered on this examination line to the vitamin C of orange juice[20]. Ascorbic acid is an important nutrient in fruit-based products and is considered a quality marker in addition to shelf life[13]. Vitamin C turned into observed to be the main contributor to antioxidant ability, accompanied by flavonoid and carotenoid compounds[21]. Increasing storage time results in a decrease in vitamin C bignay fruit juice, for the reason that is a reshuffle or oxidation of ascorbic acid [22].

TABLE I  
VITAMIN C OF BIGNAY FRUIT JUICE

| Day | Aloe-gel |        |        |
|-----|----------|--------|--------|
|     | 25%      | 50%    | 75%    |
| 0   | 176.36   | 180.71 | 145.74 |
| 3   | 132.48   | 171.62 | 134.92 |
| 6   | 88.37    | 75.29  | 73.59  |
| 9   | 98.01    | 79.47  | 57.68  |
| 12  | 73.53    | 69.96  | 53.98  |
| 15  | 69.58    | 58.17  | 56.29  |

### D. The antioxidant activity

The results showed that the addition of AVG showed a significant difference. The average antioxidant activity of bignay fruit juice is 8.69-30.82%. The highest antioxidant activity was obtained at 25% AVG treatment on day 6 (Fig. 5). AVG contains glucomannan polymer, which can bind anthocyanins in bignay fruit. Antioxidants are compounds in food that capture and neutralize free radicals, which can also play a role in coronary heart disease, cancer, and various diseases [23]. Antioxidant activity can be described as interference or inhibition of nutrient oxidation by restraining oxidative chain reactions [24]. According to [1], the antioxidant activity of ripe bignay fruit is  $14.01 \pm 0.02$ ppm. The increase in storage time resulted in a decrease in the antioxidant activity of bignay fruit juice. In general, ripe bignay can be considered a valuable source of antioxidants. Bignay AVG blend is a functional drink containing physiologically active components [11]. Besides, the antioxidant compounds in AVG can increase food's stability and nutritional value. The health benefits of AVG consist of increasing high-density lipoprotein and blood sugar in people with diabetes and boosting the immune system [25]. The above effect implies that bignay fruit juice with AVG contains bioactive compounds that can be used as a source of natural antioxidants.

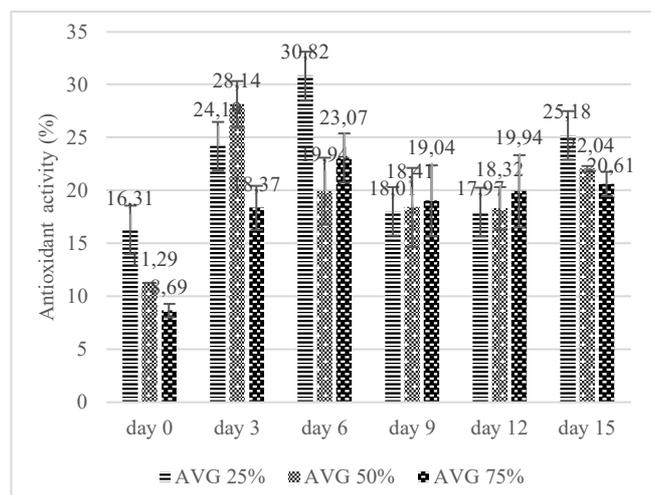


Fig. 5 Antioxidant activity of bignay fruit juice (%)

### E. Viscosity

The addition of AVG significantly affected the viscosity of bignay juice. The average viscosity value of bignay juice was 5.13-11.62 mPa.s. AVG contributed to the increase in the viscosity of bignay juice (Fig. 6). A higher AVG percentage also results in higher viscosity. Viscosity is an important concern in the beverage industry because it affects the

processing and packaging of products and can affect customer acceptance [26]. Research by [6] showed that the viscosity of AVG after extraction was high enough that it would decrease and eventually dilute or approach water viscosity after storage. Bignay extract formulation with the addition of AVG will maintain the viscosity of bignay fruit juice because the acidic environment can maintain the gel structure of AVG[27].

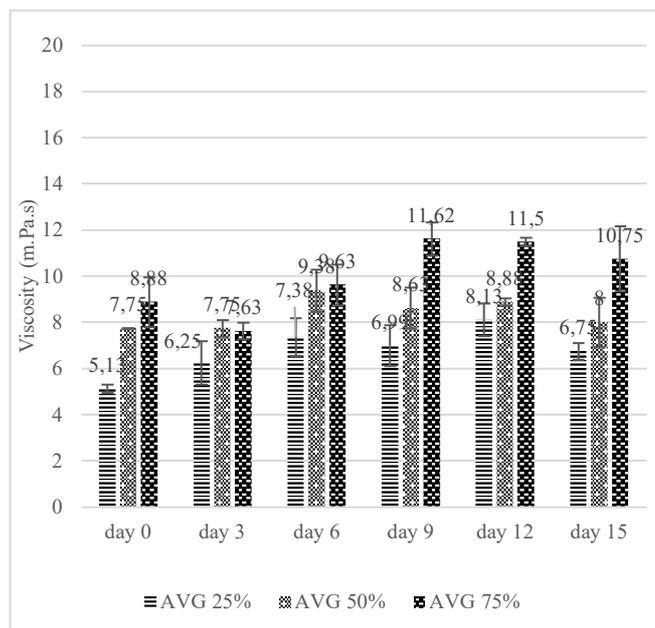


Fig. 6 The viscosity of bignay fruit juice (m.Pa.s)

#### F. Acidity

The treatment of AVG concentration did not show any difference. The acidity of bignay fruit juice is relatively stable during storage at temperatures  $6\pm 1^{\circ}\text{C}$  (Table II). Organic acids and phenols in bignay fruit are very high and contribute to the increased acidity of bignay fruit juice [14]. Bignay fruit produces a lower pH; the pH of bignay juice is about  $3.453 \pm 0.031$ . In comparison, the acidity of AVG ranges from 4.41 at room temperature [28]. Acidity values affect an emulsion's antioxidant activity [29][30].

TABLE II  
THE ACIDITY OF BIGNAY FRUIT JUICE

| Day | Aloe-gel |      |      |
|-----|----------|------|------|
|     | 25%      | 50%  | 75%  |
| 0   | 3.18     | 3.36 | 3.82 |
| 3   | 3.35     | 3.35 | 3.79 |
| 6   | 3.07     | 3.29 | 3.61 |
| 9   | 3.12     | 3.35 | 3.89 |
| 12  | 3.04     | 3.23 | 3.73 |
| 15  | 2.92     | 3.07 | 3.34 |

#### IV. CONCLUSION

The AVG affected the anthocyanin and antioxidant activity of bignay fruit juice. In conclusion, the best formula for bignay fruit juice is the addition of AVG with a concentration of 25%. The bignay fruit juice notes many functional compounds until the ninth day of storage. Bignay fruit juice can be a recommendation for health-conscious consumers to fulfill their functional drink desires.

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