



Bioactive compounds content of Snake Fruit Peel, Aloe Vera, and Stevia Extracts as Raw Material of Functional Drinks

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ABSTRACT

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The objective of the research is to analyze the content of the bioactive compounds of extracts made of snake fruit skin, Aloe vera, and Stevia as materials to make functional drinks. *Salacca zalacca* (Gaert.) Voss. cultivar has flavonoid, tannin, and a bit of alkaloid on its fruit peel. Aloe vera and Stevia also known to have antioxidant compounds. Aloe vera controls the metabolism of carbohydrates and maintaining homeostasis of glucose. The research methods are divided into steps: 1) the extraction of Snake fruit peel, Aloe vera, and Stevia; 2) the formulation of the functional drink; 3) the evaluation of antioxidant, total phenol, and tannin levels of the formulated extracts. The research used Randomized Group Design (RGD) of two factors, and each factor consists of 4 levels and 2 levels. Factor I=the ratio of snake fruit peel:Aloe vera extract (10:90 (v/v); 30:70 (v/v); 70:30 (v/v); 90:10 (v/v)) and factor II= addition of Stevia filtrate (2%; 4%) (v/v total). The best nutrient of the formulated functional drink is obtained by the composition of 90% snake fruit peel extract, 10% aloe vera, and 4% stevia. The snake fruit peel extract significantly affects the antioxidant activity and total phenol composition, aloe vera extract has an insignificant effect on active compounds, and stevia affects the tannin level of the functional drink.

1. INTRODUCTION

Snake fruit (*Salacca zalacca* (Gaert.) Voss.) is one of commodities which included in the priority list of Indonesia's original superior fruits. In Yogyakarta, snake fruit had the highest production aspect as 73283 tons in 2015. Most snake fruits are produced in Sleman (71705 tons or 97.85% of total production), and in Kulon Progo (1572 tons or 2.15% of total production) (BPS-DIY, 2015). The edible portion of snake fruits is only 56 to 65 percent, so the waste could possibly reach 35 to 44 percent of processed or consumed amount of snake fruits. Snake fruit seeds have 25 to 30 percent of portion, while the peel has 10 to 14 percent of the portion of total weight (Supriyadi *et al.*, 2002).

Snake fruits peel has flavonoid, tannin, alkaloid, hydroquinone (Sahputra, 2008), and antioxidant as ascorbate acid. The functional drink is a food product made of natural, consumable daily and has a certain function when digested in body metabolism. Snake fruit peel extract, which processed into a functional drink, expected to be an alternative of health drinks beside herb drinks, which are identical to the bitter taste and strong smell.

Other commodities which also have antioxidant and can improve overall taste are stevia and aloe vera. The snake fruit peel extract is formulated with stevia and aloe vera to get the most optimal composition of nutrients. This research is preliminary research to determine the formulation, which later will be expanded to functional drink with local commodities of Indonesia as its raw materials.

2. RESEARCH METHOD AND DESIGN

2.1. Research Sample and Steps

Materials used in the research are snake fruit peel of (*Gaert.*) *Voss*. Cultivar from Desa Turi, Sleman, Yogyakarta; Aloe vera from Tajem, Sleman, Yogyakarta; and stevia from Pasar Beringharjo, Yogyakarta; acetate buffer solution (pH 5.5), ethanol 70%, and 200 μ l DPPH solution.

The research used Randomized Group Design (RGD), which consists of two factors; each factor consists of 4 levels and 2 levels. Those factors are as follows: Factor I=the ratio of snake fruit peel:Aloe vera extract (10:90 (v/v); 30:70 (v/v); 70:30 (v/v); 90:10 (v/v)) and factor II= addition of filtrate Stevia (2%; 4%) (v/v total).

2.2. Snake Fruit Peel Extraction

The extraction method is done with the Soxhlet method, which has steps as follows:

- a) The washing of snake fruit peels
- b) The drying with direct sunlight for approximately 5 hours
- c) Size compressing by a blender to get rough and not too smooth substances
- d) Dissolution active compounds with ethanol as solvent by using *soxhlet*
- e) Evaporation, as to evaporate the ethanol as solvent from the snake fruit peel extract in temperature not higher than 80°C.

2.3. Aloe Vera Extraction

The extraction of aloe vera is done based on methods of Aji (2014), which referred to Saritha dan Khanum (2010), which is maceration by using ethanol. The Aloe vera is cleaned, is peeled from its leaf peel, then its leaf 'meat' is cut into small pieces and is blended till smooth in a juicy texture. The juice is then measured in a volumetric measuring glass.

Maceration is done at a temperature of 90°C for 17 hours. The result of maceration is then filtered by using filter paper. The filtrate and its residue are then obtained. The filtrate is then evaporated by using a rotary evaporator at 37°C to separate the methanol solvent so that the dense Aloe vera extract is obtained. The obtained Aloe vera extract is then evaluated by its total phenol, antioxidant activity, and pH levels.

2.4. Production of Infused Solution of Stevia

The Stevia is extracted by using the infusion method in mineral water, with the ratio of the mass of stevia:mineral water (1:10) b/b, heated in a stove till the temperature reaches 90°C for 15 minutes.

2.5. Measurement of Qualitative Parameters

a) Antioxidant Activity Test by using DPPH Method ((Kubo *et al.* (2002) and Molyneaux (2003))

The measurement of antioxidant activity is done with free radical DPPH (1,1-diphenyl- 2-picryl-hydrazine radical-scavenging). The snake fruit peel extracts and four chosen formulation of drinks from each treatment are used as samples of antioxidant activity test. Ascorbate acid is used as a comparative standard to the antioxidant activity contained in the formula. Therefore, the antioxidant activity level will be calculated by its equality to antioxidant activity in ppm AEAC (Ascorbic acid Equivalent Antioxidant Capacity).

b) pH solution test

The extracts of snake fruit peel, aloe vera, and stevia are tested by using pH paper indicator to get their pH level.

c) Total Phenol and Tannin Tests

As much as 5 ml of the extract is put into a test tube, then 5 drops of 10% NaCl is added. The solution is then divided into two parts in different test tubes. The first test tube is added 3 drops of FeCl_3 then is left for a while until the color changes. The change of color to black-green color indicates the presence of phenol and tannin compounds contained in the sample.

3. RESULT AND DISCUSSIONS

3.1. Bioactive compounds ingredients

3.2. Bioactive compounds ingredients of some formulated extracts

The research used eight different formulations of snake fruit peel, aloe vera, and stevia extract concentrations. Snake fruit peel and stevia, which are the main components in the functional drink with the total concentration of snake fruit peel-aloe vera extract as 100%. Each of the concentrations of snake fruit peel extract and Aloe vera has four variants, i.e., 10%, 30%, 70%, and 90%, while the concentration of stevia has two variants, i.e., 2% and 4%. Therefore, there are eight combinations of functional drink formulations observed in the research as follows:

- 1) Snake fruit peel extract 10%; aloe vera 10%; and stevia 4% (F1)
- 2) Snake fruit peel extract 30%; aloe vera 30%; and stevia 4% (F2)
- 3) Snake fruit peel extract 70%; aloe vera 70%; and stevia 4% (F3)
- 4) Snake fruit peel extract 90%; aloe vera 90%; and stevia 4% (F4)
- 5) Snake fruit peel extract 10%; aloe vera 10%; and stevia 2% (F5)
- 6) Snake fruit peel extract 70%; aloe vera 30%; and stevia 2% (F6)
- 7) Snake fruit peel extract 30%; aloe vera 70%; and stevia 2% (F7)
- 8) Snake fruit peel extract 10%; aloe vera 90%; and stevia 2% (F8)

The antioxidant activity, total phenol, and tannin level obtained from the evaluation of the extracts in the research can be seen in Table 1 below.

Table 1. Bioactive Compounds Content of Various Formulas

Variant of formulation	Antioxidant Activity (%)	Total Phenol (ppm)	Tannin (ppm)
100% Snake fruit extract	95.95	562.37	591.91
1	86.22	83.08	90.74
2	90.27	214.08	227.87
3	92.43	424.24	447.53
4	93.65	468.48	493.77
5	89.32	90.94	99.17
6	91.22	234.24	248.94
7	92.43	281.68	298.52
8	93.78	364.02	384.59

Based on the table, it can be seen that the best formula of the combination according to its antioxidant activity, total phenol, and tannin levels is the formula of 90% snake fruit peel extracts, 10% aloe vera, and 4% stevia. The formula has the highest content of tannin and total phenol. Meanwhile, the formula which has the highest antioxidant activity level is a formulation of 90% snake fruit peel extract, 10% aloe vera, and 2% stevia. The experiment result also shows that the decrease of snake fruit peel extract contained in a formula results in a significant decrease in antioxidant activity level.

3.3. The effect of snake fruit peel extract to solution's bioactive compound.

Snake fruit peel extract is a composition that causes the most significant change in bioactive compounds. Based on correlation analysis, the correlation index of snake fruit peel extract composition to antioxidant activity level is 0.96, to total phenol level is 0.98, and to tannin level is 0.98. Those values are high and prove that there is a strong correlation between snake fruit peel extract composition to the content of bioactive compounds in samples of solutions.

The change of bioactive compounds content in samples of solutions is caused by the composition of snake fruit peel extract, as seen in Table 2 below.

Table 2. Bioactive Compound Contents of Snake Fruit Peel Extract Composition Variants

Snake Fruit Peel Extract (%)	Antioxidant Activity (%)	Total Phenol (ppm)	Tannin (ppm)
10	87.77	87.01	94.955
30	90.745	224.16	238.405
70	92.43	352.96	373.025
90	93.715	416.25	439.18

Bioactive compounds content on the table is tested by using One-Way ANOVA single factor analysis. The P-value for antioxidant activity percentage with various concentrations of snake fruit peel extract is 0.04; P-value for total phenol is 0.026; P-value for tannin level is 0.024. Three mentioned P-values show significant differences because

they are less than the significance level of 0.05. The result supports previous research by Sahputra (2008), which stated that the snake fruit peel contains flavonoid compounds more than other phytochemicals and (*Gaert.*) *Voss.* cultivar has a quite high amount of tannin.

3.4. The effect of aloe vera extract to solution's bioactive compounds

The choice of aloe vera as one of the compositions in sample solutions is based on its content of flavonoid, tannin, polyphenol, and saponin (Wijaya, 2013). In the research, the aloe vera extract used in sample solutions does not affect the increase of bioactive compounds content, as seen in Table 3 below.

Table 3. The Bioactive Compound Contents in Various Concentrations of Aloe Vera extract

Aloe Vera Extract (%)	Antioxidant Activity (%)	Total Phenol (ppm)	Tannin (ppm)
10	93.715	416.25	439.18
30	92.43	352.96	373.025
70	90.745	224.16	238.405
90	87.77	87.01	94.955

Based on the table, there is no significant difference in antioxidant activity, total phenol, and tannin levels from various concentrations of aloe vera extract in samples of solutions. The correlation index shows a negative value, and the P-values are greater than the significance level of 0.05. P-value for antioxidant activity level is 0.2; P-value for total phenol is 0.11, and P-value for tannin is 0.1. Less significant effects of formulated aloe vera samples may happen due to stronger characteristics of snake fruit peel extracts and fewer variants of aloe vera extract composition. The not optimal role of aloe vera also may be happened due to the choice of extraction method, sample treatment during the research, and other technical problems.

3.5. The effect of stevia to the solution's bioactive compound

Antioxidant activity of solutions' samples also shows uptrend according to the increase of stevia concentrations of 2% and 4%. Based on the experiment result, the test result of bioactive compounds' levels according to various stevia concentrations can be seen from Table 4.

Table 4. The Bioactive Compound Contents in Various Concentrations of Stevia

Stevia (%)	Antioxidant Activity (%)	Total Phenol (ppm)	Tannin (ppm)
4	90.6425	297.47	314.9775
2	91.6875	242.72	257.805

Correlation test results show negative value for stevia concentrations to antioxidant activity level. However, the correlation index for parameters such as total phenol and tannin level is 1, which indicates strong correlations. One-Way ANOVA single factor analysis test shows that the P-value and the calculated-f do not fulfill the significant difference criteria for antioxidant activity, total phenol, and tannin levels.

3.6. Regression analysis of bioactive compounds in solutions' samples

Regression test results of antioxidant activity, total phenol, and tannin levels for a group of functional drinks with stevia concentrations as 4% and 2% are showed in Figure 1 below.

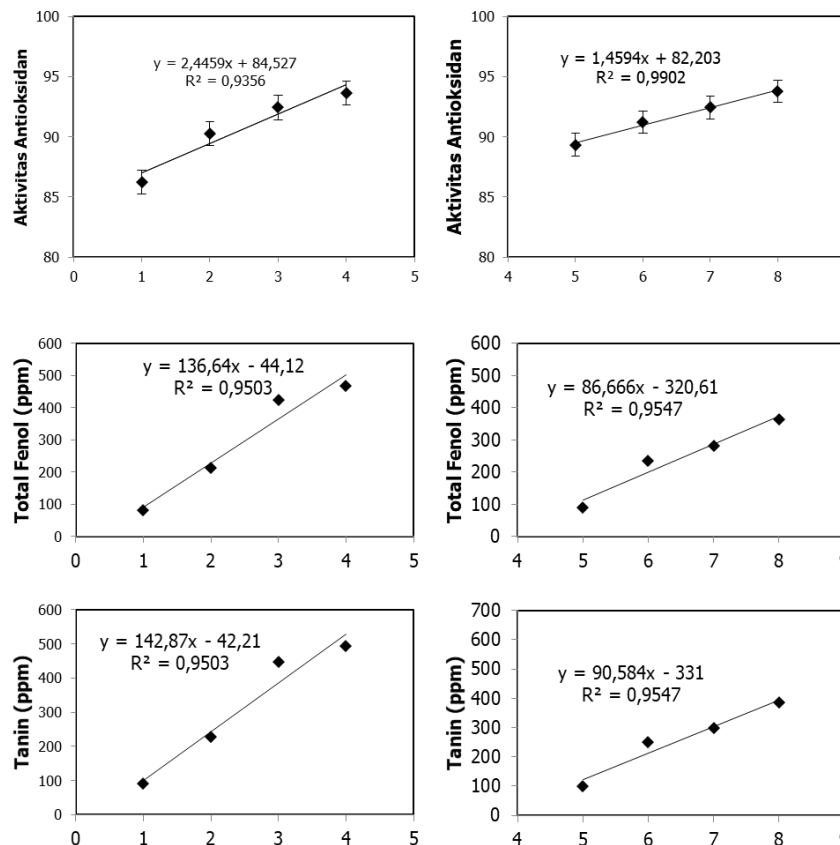


Figure 1. Regression analysis of bioactive compounds to variants of solution

From the graph, the antioxidant activity rises at the concentrations of snake fruit peel extract-alo vera 10:90%, 30:70%, 70:30%, and 90:10%. The increase is linear with the high value of R², indicates a significant increase. As in the antioxidant activity test, the total phenol contained in the functional drink keeps increasing along with the increase of snake fruit peel extract concentration. The increase is also linear with the high number of R². Tannin level in a functional drink with 2% stevia keeps increasing proportionally to the increase of snake fruit peel extract concentration linearly to the concentration of 4% stevia. The higher tannin level is shown in a functional drink with 4% stevia. Therefore, it can be considered that the concentration of stevia triggers the increase of the tannin level.

3.7. The pH of samples solutions

All formulations of functional drink in the research have pH as 5, which indicates its acidic nature. Hence, a further sensory test is needed to observe not only the health functionality but also its capability to fulfill the consumer's sensory needs. Besides, the tannin level is quite high, which may cause the functional drink to have tart or bitter taste later.

3.8. The selection of the best formulated functional drink

The selection of the best formula for a functional drink is based on the average quality of the antioxidant activity, total phenol, and tannin, as seen in Table 1. Based on Table 1, the snake fruit peel extract concentration of 100% provides the highest

antioxidant activity, total phenol, and tannin levels. However, the extract still has to be fortified with other raw materials to create a taste that consumers will like. With the formulation of snake fruit peel extract with aloe vera and stevia extracts, the best formula for its content of bioactive compounds is the formula of 90% snake fruit peel extract, 10% aloe vera, and 4% stevia.

4. CONCLUSIONS

The best formula of functional drink to have the best total phenol and tannin levels is 90% snake fruit peel extract, 10% aloe vera extract, and 4% stevia. The functional drink which has the best antioxidant activity level is the formula of 90% snake fruit peel extract, 10% aloe vera, and 2% stevia. Snake fruit peel extract has the property to increase total phenol and tannin contained in a functional drink. Meanwhile, stevia has the property to increase the antioxidant activity and tannin levels.

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