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Powder drink of *Persea americana* Mill. Seed with adding *Zingiber officinale* Rosc. Var Rubrum and *Stevia rebaudiana* L. to enhance Cardiovascular health

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ABSTRACT: In today's modern era many people consume unhealthy foods, which can lead to decreased immunity. One way to increase immunity is by consuming functional drinks from Avocado seeds and red ginger, which is contain flavonoid compounds. This study aims to determine antioxidant activity in functional drinks of avocado seeds by adding red ginger and stevia. The method used in this research is a quantitative analysis using the UV-Vis Spectrophotometer instrument at a wavelength of 418 nm for F1, F2 and F3 were brown, had a characteristic smell of spices, and had a distinctive taste. The pH test results were 6.243 ± 0.025, 6.167 ± 0.045, and 6.089 ± 0.005 respectively. The raw material and functional drinks positively contain flavonoids. Flavonoid content in F1, F2, and F3 were 12.214 ± 0.009428%, 12.419 ± 0.009428%, and 12.624 ± 0.009428% respectively. Antioxidant test results for functional drinks showed an IC_{50} value of F1 and F2 were 118.30 ± 1.57 ppm and 105.88 ± 1.11 ppm, which is categorized as moderate levels of antioxidants, and F3 was 88.09 ± 1.62 ppm, which is categorized as strong levels of antioxidants.

KEYWORDS: Antioxidant activity; avocado; functional drink; red ginger; stevia.

INTRODUCTION

Health is important in relation to one's productivity [1]. Health Law Number 36 of 2009 provides a limitation: health is a state of physical, mental, and social well-being that enables everyone to live productively socially, and economically [2]. The simplest way to maintain health is to consume functional drinks that are good for health [3].

Indonesian Food and Drug Authority (BPOM) in 2011 stated that functional food is food that naturally or has gone through a process, and contains one or more compounds that based on scientific studies are considered to have certain physiological functions that are beneficial to health, one of which is is a functional powder drink[4]. Functional drinks can be derived from natural ingredients that are easily found in vegetable ingredients, including avocado (*Persea americana* Mill.) [5], proves that are rich in antioxidants and nutrients [6].

The production of avocados in Central Java has increased every year. With 44,522 tons produced in 2018 and an increase of 60,145 tons in 2019, products of seeds that have not been utilized optimally will be produced [7]. Even though the content of antioxidants, fiber, and phenolic compounds in avocados is more concentrated in the seeds of the fruit than in the flesh [5]. According to [8], stated that avocado seeds contain secondary metabolites including flavonoids, steroids, terpenoids, saponins, tannins, cardiac glycosides, and alkaloids. Avocado seeds have antioxidants with an IC_{50} value of 31.5 ppm [9]. Avocado seeds have a bitter taste, so they need to be combined with other ingredients such as red ginger to give the drink formulation a taste and aroma [10].

One of the most abundant commodities in Indonesia is red ginger (*Zingiber officinale* var Rubrum), and it is still less in application. It is an herb plant that has high antioxidant activity. Red ginger (*Zingiber officinale* var Rubrum) contains compounds that have the potential to be antioxidants, such as phenols and flavonoids. Red ginger (*Zingiber officinale* var Rubrum) has antioxidant activity with an IC_{50} value of 56.846 µg/mL. Antioxidants can control blood glucose levels, improving pancreatic function in producing insulin [11].

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The term "antioxidant" actually does not refer to one particular compound but the activity of certain compounds in the body. There are many types of antioxidants, including several antioxidant vitamins, minerals, and polyphenols. Combining 2 or more ingredients with a high enough antioxidant content will result in the best blend of antioxidants, making it easy to maximize health benefits. These antioxidant compounds include lycopene, carotenoids, lutein, zeaxanthin, anthocyanins, quercetin, glutathione and flavonoids [12].

Functional drinks tested based on physical quality include those that pass organoleptic, pH, and antioxidant activity tests [13]. Indonesian National Standard number 01-4320-1996 explains the physical requirements of traditional powder drinks: color, smell, and taste are normal, typical of spices, and the type of spices [14]. One of the components of functional drinks that have physiological functions for the body are antioxidants [15]. Measurement of antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazil) method is a simple method that uses a small number of samples in a short time [16].

Based on the description above, the author wants to utilize avocado seeds waste, so it can be processed into drink products with the addition of red ginger and stevia as a flavor, fragrance, and sweetener which is made into a functional powder drink that is efficacious as an antioxidant. As a result, the authors are intrigued by the study titled "Formulation Functional Drink of Avocado (*Persea americana* Mill.) Seeds with Adding Red Ginger (*Zingiber officinale* var Rubrum) and Stevia (*Stevia rebaudiana* L.)"

▪ MATERIALS AND METHODS

Materials

The main raw materials used were the simplisia of avocado (*Persea americana* Mill.) seeds, red ginger *Zingiber officinale* var Rubrum) and stevia (*Stevia rebaudiana* L.), which were obtained from Yogyakarta. Quercetin, $AlCl_3$ 10%, DPPH (2.2-diphenyl-1-pikrilhydrazil) were obtained from Sigma (Sigma Aldrich GmbH, Sternheim, Germany). Acetic acid, Mg powder, HCl 37%, and methanol p.a. were obtained from Merck, Darmstadt, Germany. Whatmann 41 filter paper, aquades, pH 4, 7, and 10 buffers.

The equipment for this research was analytical balance (Labex, London, UK), 18-mesh sieve, blender (Fomac-Miller Machine FCT-Z500, China), pH meters (Hanna HI 8010, USA), beaker glass (Iwaki Pyrex, Indonesia), magnetic stirrer, hotplate stirrer (Thermo scientific, Boston, USA), thermometers, drip pipettes, test tubes (Iwaki Pyrex, Indonesia), tube racks, vortex (Thermo scientific, Boston, USA), spatula, volumetric glass (Iwaki Pyrex, Indonesia), glass funnels (Iwaki Pyrex, Indonesia), volumetric pipettes (Iwaki Pyrex, Indonesia), volumetric flask (Iwaki Pyrex, Indonesia), cuvettes (Purshee, USA), and UV-Vis spectrophotometers (Raptor, USA). This research is of the descriptive type with a quantitative descriptive design because it describes a sample objectively with data in the form of numbers or shaken data, and the variables used in this study are single variable.

Powder drinks of *Persea americana* Mill. seeds with adding *Zingiber officinale* var Rubrum and *Stevia rebaudiana* L.

This study was carried out in three stages: the first was the production of functional drink powder, the second was the testing of functional drink powder, and the third was the processing and analysis of the data collected. Determination of avocado (*Persea americana* Mill.) seed: the determination of avocado (*Persea americana* Mill.) seeds was carried out in the Pharmacy Biology Laboratory, Islamic University of Indonesia. Extraction of avocado seeds and red ginger refers to [13] modification which is the simplisia powder of avocado seeds and red ginger are each infused with aquadest (1:10) at a temperature of 90 °C for 15 minutes and strained, then evaporated until thick. The composition of functional powder drinks can be seen as follows.

Table 1. Functional powder drinks composition.

Composition	Formula (%)		
	1	2	3
Avocado seeds	40	35	30
Red ginger	30	35	40
Stevia	30	30	30

The process of functional powder drinks refers to the modification of [17] , extract of avocado seeds and red ginger mixed according to treatment, and stevia added to the juice mixture. The mixture of stevia and extract is heated over low heat and maintained at a constant temperature of 50°C while continuing to stir until crystals form. The resulting crystals were crushed with a blender and sieved through an 18 mesh sieve to obtain a functional drink powder, which was then used for further analysis.

Powder drinks of *Persea americana* Mill. seeds with adding *Zingiber officinale* var Rubrum and *Stevia rebaudiana* L. quality test

Organoleptic test

Organoleptic testing is a means of testing using the human senses as the primary tool for measuring the power of reception to products that include smell, color, taste, and texture [18].

pH test

Measured pH sample using pH meters that have been calibrated first using a pH buffer of 4, 7, and 10 then the sample of 8 grams is dissolved in 20 mL aquades, then dipped the pH meter electrode into the sample and waited until the reading number becomes stable. The pH measurement is replicated three times and calculated on average [19].

Qualitative test of flavonoid powder of raw material and Powder Drinks of *Persea americana* Mill.) seeds with adding *Zingiber officinale* var Rubrum and *Stevia rebaudiana* L.

A total of 4 grams of the sample and 0.4 grams of quercetin as a comparison are dissolved in 40 mL of methanol p.a., then 10 mL are taken and put into a test tube. Added to the sample and comparison in the form of Mg powder (2 mg) and given 3 drops of concentrated HCl. The sample and comparison is shuffled, and changes that occur are observed. Tests were conducted with three replications, and the formation of red, yellow, or orange in the solution showed the presence of flavonoids [15].

Quantitative test total flavonoid levels powder drinks of *Persea americana* Mill. seeds with adding *Zingiber officinale* var Rubrum and *Stevia rebaudiana* L.

The manufacture of a standard solution of 200 ppm quercetin is done by weighing as much as 10 mg of quercetin, dissolving it in methanol p.a. to taste, and adding 50 mL of takar squash until it is homogeneous [9]. Determination of the maximum wavelength of quercetin is done with a standard solution of 200 ppm quercetin, which is then made into a 100 ppm quercetin solution. A total of 1 mL of 100 ppm quercetin solution, 1 mL of $AlCl_3$ 10%, and 8 mL of 5% CH_3COOH were added. The solution is incusted for 30 minutes, and absorbance is measured at wavelengths of 350–500 nm [20].

The manufacture of the calibration curves will be done by making serial solution levels of 40, 60, 80, 100, and 120 ppm. A total of 1 mL of serial solution levels of each concentration are entered, and they react with 1 mL of $AlCl_3$ 10% and 8 mL of CH_3COOH 5%. After 30 minutes, the sample was silenced, and a series of absorption readings of levels were taken using UV-Vis spectrophotometry at a maximum wavelength [21].

Determination of total flavonoid levels in functional drink samples has been done by making a concentrated solution of 800 ppm, then spinning a vortex for 10 minutes at a speed of 3000 rpm. Then take much as 1 mL sample is inserted into a test tube and added 1 mL $AlCl_3$ 10% and 8 mL CH_3COOH 5%. Then the sample is incubated for 30 minutes, with absorbance measured at the maximum wavelength [21].

Flavonoid levels are calculated using linear regression equations based on calibration curves resulting from UV-Vis spectrophotometer readings. Absorbance data obtained from measurements are entered into linear regression equations as y and x values representing raw solution concentrations. The linear regression equation is expressed by the formula: y = bx + a, where y = absorbance, a = interception, x = concentration (ppm), and b = slope (slope).

The absorbance results of the sample measurement are entered into linear regression. Absorbance of the sample as y, so that the total flavonoid levels obtained are expressed as the mg amount equivalent of quercetin (QE) in each gram of the sample [21]:

$$flavonoids\ content = \frac{x}{mg\ sampel} \times \frac{vol\ add}{1000\ mL} x\ 100\%$$

Antioxidant activity powder drinks of *Persea americana* Mill. seeds with adding *Zingiber officinale* var Rubrum and *Stevia rebaudiana* L.

The test is carried out by piping a number of methanol extract sample stock solution of 1000 mg/L, then adding 1.0 mL DPPH 0.4 mM and then 5.0 mL of methanol are sufficient. The solution is allowed to stand for 30 minutes at room temperature. The absorption was measured with a UV-Vis spectrophotometer at a wavelength of 516.5 nm. Antioxidant activity is determined from the IC_{50} value.

The IC_{50} value is a number that indicates the concentration of the test sample that provides immersion of 50% (able to inhibit or soak the oxidation process by 50%). The value of IC_{50} is determined by making a linear curve between the concentration of the test solution (x-axis) and % inhibition (y-axis) so that the equation y = bx + a where y is % inhibition and x is the value of IC_{50} [22].

$$IC_{50} = \frac{50-a}{b}$$

RESULTS

Determination of avocado (*Persea americana* Mill.) seed

The first step in this research is to determine the sample used, namely avocado seeds. This determination is carried out macroscopically at the Pharmacy Biology Laboratory of the Islamic University of Indonesia. The determination aims to ensure and prove that the identity of the sample used in this study is true avocado (*Persea americana* Mill.) seed. The result of the determination is obtained by the formula:

1b-2b-3b-4b-7b-9b-10b-11b-12b-13b-14b-16a-(class 10)

239b-243b-244a-245b-246b-247a-(Lauraceae)

1a-2a-13a-14a-(*Persea americana* Mill.)

Organoleptic and pH tests of powder drinks

Table 2. Organoleptic test of powder drinks.

Test Criteria	Result of formula		
	1	2	3
Color	Browniest white	Browniest white	Browniest white
Smell	Distinctive spices	Distinctive spices	Distinctive spices
Taste	Distinctive spice, sweet	Distinctive spice, sweet	Distinctive spice, sweet
Texture	Fine powder	Fine powder	Fine powder

Table 3. pH test of powder drinks.

Formula Functional Powder Drinks	Replicaton			Mean±SD	Category
	1	2	3		
F1	6.21	6.23	6.09	6.243±0.025	Weak acid
F2	6.27	6.14	6.09	6.167±0.045	Weak acid
F3	6.25	6.13	6.08	6.089±0.005	Weak acid

Qualitative test of flavonoid on raw material and powder drinks

Table 4. Flavonoid qualitative test.

Sample	Color	Interpretation
Flavonoid standard	Red	+
Avocado seed powder	Red	+
Red ginger powder	Red	+
Stevia powder	Red	+
F1	Red	+
F2	Red	+
F3	Red	+

Total flavonoid test on powder drinks

Table 5. Result of total flavonoid levels powder drinks.

Formula	Replication	Absorbance	Concentration (ppm)	Total Flavonoid level (%)
F1	1	0.625	97.771	12.221
	2	0.625	97.771	12.221
	3	0.624	97.607	12.201
	Mean±SD	**0.625**	**97.716**	**12.214±0.009428**
F2	1	0.635	99.409	12.426
	2	0.634	99.246	12.406
	3	0.635	99.409	12.426
	Mean±SD	**0.635**	**99.355**	**12.419±0.009428**
F3	1	0.645	101.049	12.631
	2	0.644	100.885	12.611
	3	0.645	101.049	12.631
	Mean±SD	**0.645**	**100.994**	**12.624±0.009428**

Antioxidant activity on powder drinks

Table 6. The antioxidant activity of powder drinks.

Formula	Replication	IC_{50} (ppm)	Mean±SD	Category
F1	1	119.80	118.30±1.57	Moderate
	2	116.67		
	3	118.43		
F2	1	106.42	105.88±1.11	Moderate
	2	104.61		
	3	106.61		
F3	1	89.83	88.09±1.62	Strong
	2	87.82		
	3	86.61		

▪ DISCUSSION

The determination was made by adjusting the morphological condition of the plant using the key of determination contained in the Javanese flora [23]. From the results of the determination formula, it can be ascertained that the plant is *Persea americana* Mill., commonly known as avocado.

The stage of making a functional powder drinks begins with weighing the powders of avocado (*Persea americana* Mill.) seeds and red ginger (*Zingiber officinale* var Rubrum). It is suggested that the infusion or extract of the sample be made by heating the material and aquadest solvent in a ratio of 1:10, then heated at 90°C for 15 minutes using a water bath. The extract obtained is then blown up to half its initial volume, and the powder is made by mixing stevia (*Stevia rubidiana* L.) and stirring until it crystallizes. Selection of the extraction ratio of 1:10 because this comparison is the best against flavonoid compounds [24].

The addition of sucrose is intended as a sweetener and to form a crystallization process made into drink powder to improve quality and facilitate storage so that its functional properties for health can be well maintained [25]. The crushed crystals were then sieved with an 18 mesh sieve because the degree of fineness produced was higher so that the powder dissolved in water more quickly, suggesting that the degree of fineness indicates the uniformity of the milling results or the distribution of coarse and fine fractions. The finer the powder, the faster it will dissolve in water because the surface of the powder that is in direct contact with the solvent is getting wider, while the coarser the powder, the longer it will take to dissolve because the more cells the solvent has to penetrate [13].

The first test that was performed was an organoleptic test. Organoleptic tests were carried out to determine the physical quality of functional powder drinks, including color, smell, taste, and texture, with the results shown in Table 2. The results obtained are in accordance with SNI 01-4320-1996 regarding the

quality standard of traditional powder drinks, which explains that traditional powder drinks must have a normal color, smell, and taste typical of spices [14].

The pH test on functional powder drinks is performed using a pre-calibrated pH meter. Calibration is part of the maintenance of the tool and aims to ensure that the measurement results of the tool are acceptable and fall within the required validation range. Calibration of pH is carried out using a standard buffer solution reference material at acidic, alkaline, and neutral conditions [26].

The pH test is a standard of acidity that determines the quality of a functional powder drink after being dissolved in water. The pH of the functional powder drink depends on the type and amount of raw materials added during the manufacturing process. The results are in accordance with the research of [27], who found that the pH test results for powdered drinks must be acidic (pH 6–6.8) because it can affect the taste quality of the functional powder drinks.

Test results from three formulas of functional powder drinks showed that these drinks had weak acidic properties due to the presence of flavonoid content in them. It is suggested that flavonoids are a group of polyphenols, so they have the chemical properties of phenol compounds that are acidic so they can dissolve in bases and have antioxidant properties [28].

Qualitative tests of flavonoids are conducted to determine the presence of flavonoids in functional powder drinks. The results of the flavonoid qualitative test can be seen in Table 4. Qualitative test results of flavonoids on avocado (*Persea americana* Mill.) seeds powder, red ginger (*Zingiber officinale* var Rubrum) powder, stevia (*Stevia rebaudiana* L.) powder, and functional powder drinks of avocado (*Persea americana* Mill.) with adding red ginger (*Zingiber officinale* var Rubrum) and stevia (*Stevia rebaudiana* L.) are proven by the formation of a red color in the solution. It is said to be positive if it contains flavonoids in accordance with the research of [29], which states positive results if a red or orange solution is formed, indicating the presence of flavonoids.

Quantitative tests of total flavonoids were conducted to determine the levels of flavonoids in functional powder drinks. The results of the total flavonoid quantitative test can be seen in Table 5. Total flavonoids are the total amount of secondary metabolite compounds derived from a plant. The total content of flavonoids is measured based on the presence of quercetin in plant extracts because quercetin is the most active substance in flavonoids, so quercetin represents other flavonoid compounds [30]. Quantitative tests of total flavonoids were conducted with a UV-Vis spectrophotometer at a maximum wavelength of 418 nm. The wavelengths used for quantitative tests of flavonoids total between 350-500 nm [20].

Long maximum waves are done to find out at what wavelength there is the maximum absorption value in the sample, so that the measurement results are accurate and minimize errors [27]. The solvent used is methanol p.a. because methanol is polar. Methanol is a liquid that easily enters the cell through the material's cell wall, so that the metabolites of the sunder contained in the cytoplasm will be dissolved in the solvent and the compound will be extracted perfectly.

Flavonoid compounds are polar compounds because they have a number of bound sugars that are bound, therefore, flavonoids are more likely to dissolve in polar solvents. According to the principle of polarization, a compound will dissolve in a solvent that has the same polarity [31]. Quantitative tests of total flavonoids were conducted to look for flavonoid levels in functional drink samples measured quantitatively by the aluminum chloride method using a standard solution of quercetin. Quantitative tests of total flavonoids were conducted with the addition of 1 mL of 10% $AlCl_3$ and 8 mL of acetic acid for every 1 mL of sample solution concentration to be tested [30], suggested that the total flavonoid content was determined based on a colorimetric reaction, that is, after the sample was reacted with $AlCl_3$ in an acidic medium. The addition of $AlCl_3$ in the sample can form a complex between aluminum chloride and quercetin, resulting in a shift in wavelength towards visible (visible) and characterized by the solution producing a more yellow color. The function of adding acetic acid is to maintain wavelengths in visible areas [32].

Samples containing flavonoids will react with the $AlCl_3$ reagent to form a reaction complex between hydroxyl groups and neighboring ketones or with neighboring hydroxyl groups. Solution of $AlCl_3$ will react with ketone groups in C-4 and OH groups on C-3 or C-5 in flavon compounds or flavonols to form stable yellow complex compounds. Compounds used as standards in determining flavonoid levels are quercetin, because quercetin is a flavonoid flavonol group that has a keto group on C-4 atoms and also a hydroxyl group in neighboring C-3 and C-5 atoms [21]. Based on the results of these measurements, the higher the concentration, the higher the absorbance. Linear regression equation obtained, i. e y = 0.0061x+0.0286. The

average flavonoid total content of functional powder drink of avocado (*Persea americana* Mill.) with adding red ginger (*Zingiber officinale* var Rubrum) and stevia (*Stevia rebaudiana* L.) that can be seen in Table 5. Based on these data, the more addition of red ginger (*Zingiber officinale* var Rubrum) powder adder, the higher the total flavonoid contents, because the content of flavonoids in red ginger (*Zingiber officinale* var Rubrum) is higher than avocado (*Persea americana* Mill.) seeds.

The antioxidant activity test was conducted to determine the antioxidant properties in functional powder drinks. Functional powder drinks samples have flavonoid content that has the potential to be used as antioxidants. The antioxidant activity of functional drinks is expressed as inhibitory concentration 50 (IC_{50}), which is defined as the concentration of antioxidant compounds needed to reduce free radical activity by 50%, where the smaller the IC_{50} value, the higher the antioxidant activity. Determination of antioxidant activity is done by the method of DPPH (2,2-diphenyl-1-picrylhydrazyl) by utilizing DPPH free radical compounds in polar solvents such as methanol to test antioxidant compounds in dampening free radicals [33].

The capture of DPPH free radicals by antioxidants will cause a reduction of DPPH compounds, causing the purple color to fade and the yellow diphenilpycrilhydrazine complex to form, which is non-radical [34]. Based on the results, the IC_{50} value of a functional powder drinks with Formula 1 and 2 are at a moderate level, and Formula 3 is at strong level based on the classification of antioxidants [21].

The moderate-level antioxidant activity possessed is suspected because the compounds contained are flavonoids of the flavonoid class. Flavonols generally have medium antioxidant activity [35]. Moderate level antioxidant activity of flavonols compounds are generally caused by hydroxyl groups contained in the structure of the compound only a little. The strong level of antioxidant activity is thought to be due to the presence of other antioxidant compounds besides flavonols [11]. From these results it can be seen that the more red ginger (*Zingiber officinale* var Rubrum) is added, the antioxidant activity increases. This is because the content of antioxidant compounds in red ginger (*Zingiber officinale* var Rubrum) is higher than avocado (*Persea americana* Mill.) seeds [8], [11] .

▪ CONCLUSION

Based on this research, it can be concluded that the organoleptic test of functional powder drinks of avocado (*Persea americana* Mill.) with adding red ginger (*Zingiber officinale* var Rubrum) and stevia (*Stevia rebaudiana* L.) had a brownies white color, distinctive spice smell, distinctive spice and sweet taste, and a fine powder texture. The average pH tests of functional powder drinks of avocado (*Persea americana* Mill.) with adding red ginger (*Zingiber officinale* var Rubrum) and stevia (*Stevia rebaudiana* L.) was 6.243±0.025, 6.167±0.045, and 6.089±0.005 respectively. Powder of avocado (*Persea americana* Mill.) seeds, red ginger (*Zingiber officinale* var Rubrum), stevia (*Stevia rebaudiana* L.) and functional powder drinks of avocado (*Persea americana* Mill.) with adding red ginger (*Zingiber officinale* var Rubrum) and stevia (*Stevia rebaudiana* L.) are contain positive flavonoid compounds, the total flavonoid content of the functional powder drinks of avocado (*Persea americana* Mill.) with adding red ginger (*Zingiber officinale* var Rubrum) and stevia (*Stevia rebaudiana* L.) was 12.214±0.009428%, 12.419±0.009428%, and 12.624±0.009428% respectively. The IC_{50} value of functional powder drinks of avocado (*Persea americana* Mill.) with adding red ginger (*Zingiber officinale* var Rubrum) and stevia (*Stevia rebaudiana* L.) Formula 1 and 2 are 118.30±1.57 ppm and 105.88±1.11 ppm, which is included in the moderate level antioxidant, and Formula 3 is 88.09±1.62 ppm, which is included in high level antioxidant.

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