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Determination Of Polyphenol Levels And Antioxidant Activity Extract Combination Instant Powder Preparation Katuk Leaf (*Sauropus androgynus* (L.) Merr.) and Cacao Beans (*Theobroma cacao* L.)

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ABSTRACT

Background: Natural immunomodulators are obtained from herbal plants with natural antioxidant content. The content of polyphenol compounds as natural antioxidants in katuk leaves and cocoa beans is utilized in the form of instant powder preparations.

Aim: This study aims to determine the levels of polyphenols and the antioxidant activity of instant powder combination of katuk leaves and cocoa beans.

Method: This study used a quantitative descriptive method with test parameters including organoleptic, moisture content, ash content, polyphenol qualitative, polyphenol content and antioxidant activity

Result: The results of the instant powder moisture content test were $1.71 \pm 0.04\%$; $1.68 \pm 0.03\%$; and $1.59 \pm 0.03\%$. The results of the instant powder ash content test were $1.16 \pm 0.03\%$; $1.18 \pm 0.04\%$; and $1.11 \pm 0.03\%$. The instant powder contained polyphenols as indicated by the formation of blackish-brown color. Polyphenol contents in instant powder were 43.83 ± 1.76 mg GAE/g; 34.40 ± 0.71 mg GAE/g; and 29.50 ± 0.76 mg GAE/g. The instant powder is classified as a medium antioxidant category with an IC_{50} value of 119.25 ppm; 129.25 ppm; and 146,40 ppm, respectively.

Conclusion: Formula 1 containing 10% katuk leaves extract and 30% cocoa bean extract is a formula with the largest polyphenol content and the strongest antioxidant activity.

Keywords: polyphenols, antioxidants, instant powders, katuk leaves, cocoa beans

INTRODUCTION

The rapid spread of the virus requires people to take several preventive measures. Viral infections are generally self-limiting diseases that can heal by strengthening the body's immunity. Increasing the immune system may prevent body from various diseases. The immune system can be maintained and enhanced by consuming vitamins and herbs from nature which may also acts as immunomodulators. Immunomodulator is a substance that can stimulate the immune system thereby increasing the activity of the immune system in fighting infection or disease ¹. Natural immunomodulators are obtained from herbal plants with natural antioxidant content ².

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Antioxidants are a group of chemicals that protect biological systems against the potential harmful effects of oxidation processes or reactions ³. Natural antioxidants commonly contained in plants are polyphenolic compounds ⁴. Polyphenols as antioxidants protect cells and body chemicals against damage caused by free radicals and reactive atoms that contribute to tissue damage in the body ⁵. Some sources of antioxidants that come from nature are katuk plants and cocoa beans. Katuk plants (*Sauropus androgynus* (L.) Merr.) contain secondary metabolites such as flavonoids and polyphenols which can have potential as antioxidants ⁶. Cocoa (*Theobroma cacao* L.) is also a type of plant that contains polyphenolic compounds which can act as antioxidants and also have potential as natural dyes ⁷.

MATERIAL AND METHODS

This research is a type of quantitative research using a descriptive and quantitative methods. The research aims to determine polyphenol levels and antioxidant activity of instant powder preparations combined with extracts of katuk leaves (L.) Merr.) and cocoa beans (*Theobroma cacao* L.). The materials used in this research were katuk leaf powder (*Sauropus androgynus* (L.) Merr.), cocoa bean powder (*Theobroma cacao* L.), 70% ethanol, 96% ethanol, ethanol p.a, sucrose, maltodextrin, aquadest, gallic acid, Folin-Ciocalteus reagent, 1% FeCl₃, 7% Na₂CO₃, and 1,1-Diphenyl-2-Pikrylhidrazil (DPPH). The tools used in this research are oven, blender, analytical balance, measuring cup, beaker, stirring rod, glass funnel, porcelain cup, 60 mesh sieve, beaker, stove, desiccator, stopwatch, test tube, spatula, 5 mL measuring flask, 10 mL measuring flask, 25 mL measuring flask, 50 mL measuring flask. This research was conducted at the Integrated Laboratory of Campus III Health Polytechnic, Ministry of Health, Surakarta in January - February 2023.

Katuk leaf powder (*Sauropus androgynus* (L.) Merr.) and cocoa bean powder (*Theobroma cacao* L.) was obtained from the Rumah Rempah Lestari Klaten. The extracts of katuk leaf and cocoa bean were made by weighing 400 g each of katuk leaf powder and cocoa bean powder in a beaker, then added with 70% ethanol (1:10) w/v. The mixture was put into a sonicator with a frequency of 50 Hz at 40°C for 45 minutes. The filtrate was filtered with filter paper, then evaporated using a water bath at 50°C.

The instant powders were made using formula (Table 1), resulting the total weight of 50 gram in each formula. All ingredients are weighed according to the formula. The condensed extract of katuk leaves, extract of cocoa powder, and maltodextrin were mixed in a baking dish until well blended. Then it was heated in an oven at 50°C for 1 hour. After the drying was complete, sucrose was added and stirred until homogeneous. Size reduction was carried out using a blender, then sifted using a 60 mesh sieve and packed in a tightly closed plastic clip ⁶. After the instant powder was obtained, physical quality tests were carried out including organoleptic tests, moisture content tests, ash content tests, and polyphenol qualitative tests using the color reaction method. Quantitative determination was carried out using the spectrophotometric method for polyphenol levels and antioxidant activity.

Table 1. Instant Powder Composition

Material	FI (%)	FII (%)	FIII (%)
Extract of katuk leaves	10	20	30
Extract of Cocoa bean	30	20	10
Sucrose	40	40	40
Maltodextrin	Ad 100	Ad 100	Ad 100

Source: (Modified by Zaddana et al., 2021; Yuliani et al., 2020⁸)

Organoleptic test was carried out on instant powder preparations which including taste, color and aroma ⁸. Moisture content evaluation was carried out with the following procedure. A total of 2 g of sample was weighed into a porcelain cup, dried in an oven at 105°C for 3 hours, cooled in a desiccator, and weighed until a constant weight was obtained ⁹. The ash content was determined by putting an empty crucible in the oven for 30 minutes at 105°C. Then, it was placed in a desiccator for 20 minutes, weighed periodically until constant. A total of 2 g of sample was weighed into a crucible, then placed on a stove at a stable temperature until the sample turned white-gray for 4 hours. The crucible is cooled in a desiccator for 1 hour and weighed ¹⁰.

Polyphenol qualitative testing was conducted using the color reaction method. As much as 1 gram of sample were dissolved with 2 mL of distilled water. Then, 3 drops of 1% FeCl₃ solution were added ¹¹. If the sample was positive, the color will change to black-brown, black-blue, or black-green ¹².

Quantitative testing of polyphenol levels was carried out by preparing a standard gallic acid solution. A standard gallic acid solution of 1000 ppm was made by weighing 10 mg of gallic acid and dissolved in 96% ethanol to a volume of 10 mL ¹³. After that, a series of gallic acid standard solutions was prepared by pipetting as much as 2.5 ml of 1000 ppm gallic acid then diluted with 96% ethanol to a volume of 25 mL, resulting concentration of 100 ppm. Series of gallic acid with concentration of 10, 20, 30, 40, and 50 ppm were prepared from gallic acid 100 ppm. As much as 1.5 mL of Folin-Ciocalteau reagent was added to each series of concentration, shaken, and left for 3 minutes. Then 1.2 mL of 7% Na₂CO₃ solution was added, shaken until homogeneous and allowed to stand for 60 minutes at room temperature. The standard concentration of 30 ppm is used for scanning the maximum wavelength. After obtaining the absorption of each series, a calibration curve was made to depict the relationship between gallic acid concentration (mg/L) and absorbance ¹⁴.

The sample solution was prepared by weighing 10 mg of sample and then dissolved with 10 mL of 96% ethanol to obtain a sample solution with a concentration of 1000 ppm. Then 1 mL of 1000 ppm sample solution was pipetted, then 1.5 mL of Folin-Ciocalteau reagent was added, shaken and allowed to stand for 3 minutes, then 1.2 mL of 7% Na₂CO₃ solution was added, shaken until homogeneous and allowed to stand for 60 minutes at room temperature. The absorption measurement at the maximum absorption wavelength was repeated 3 times so that the polyphenol levels obtained were obtained as mg gallic acid equivalent/g extract ¹⁴.

Tests for antioxidant activity were carried out by preparing a 50 ppm DPPH solution, weighing 2.5 mg of DPPH powder dissolved with ethanol p.a in a 50 mL measuring flask to the boundary mark to obtain a 50 ppm DPPH solution ¹³. The control solution was prepared by pipetting 2 ml of ethanol

p.a into a test tube and adding 2 ml of 50 ppm DPPH solution, then shaking until homogeneous. Then the solution was incubated for 30 minutes at 37°C and the maximum wavelength was measured ¹⁵. The antioxidant activity of gallic acid standard was carried out by pipetting 2 mL of standard solution from each of 5 concentration series of 10, 20, 30, 40, and 50 ppm, then adding 2 mL of 50 ppm DPPH solution, homogenizing, and incubating for 30 minutes at 37°C. Absorption was measured at the maximum wavelength. Sample was weighed as much as 5 mg, dissolved with ethanol p.a in a 5 ml measuring flask. The volume of the solution was made up with ethanol p.a until the mark limit, so that a 1000 ppm stock solution was obtained. Stock solution was pipetted 0.05 mL; 0.1 mL; 0.15 mL; 0.2 mL; and 0.25 mL and put into different 5 mL measuring flask. Ethanol p.a was added to each flask up to the mark, so that concentrations of 10, 20, 30, 40, and 50 ppm were obtained ¹³. As much as 2 mL of sample solution from each of the 5 concentration series of 10, 20, 30, 40, and 50 ppm were put it in a test tube, added with 2 mL of 50 ppm DPPH solution, then shaken until homogeneous. The solution was incubated for 30 minutes at 37°C and its absorbance was measured at the maximum wavelength ¹³.

RESULTS

The extraction results of the sonication method used 70% ethanol solvent. The ratio used between the sample and the solvent is 1:10. The yield of extraction process is presented in Table 2.

Table 2. Extraction Results of Katuk Leaf Powder and Cocoa Bean Powder

Sample	Powder weight (g)	Extract weight (g)	Yield (%)
Katuk leaves	400	110.6045	27.65
Cocoa beans	400	66.4176	16.60

The results of the organoleptic test of instant powder in Formulation I has a characteristic odor of cocoa, while Formulations II and III have a distinctive odor of katuk leaves. The colors and flavors of the three formula were dark brown, brown, slightly yellowish brown with a slightly sweet taste, respectively.

Table 3. Instant Powder Organoleptic Test Results

Sample	Flavour	Colour	Odor
FI	Little sweet	Dark brown	Cocoa special
FII	Little sweet	Chocolate	Typical of katuk leaves
FIII	Little sweet	Slightly yellowish brown	Typical of katuk leaves

Table 4. The Result of Moisture Contents on Instant Powder

Sample	Replication			Mean water content \pm SD	SNI standard
	1	2	3		
FI	1.66	1.70	1.75	1.71 \pm 0,04	< 3.0
FII	1.72	1.65	1.69	1.68 \pm 0,03	
FIII	1.63	1.58	1.56	1.59 \pm 0,03	

Table 5. The Result of Ash Content on Instant Powder

Sample	Replication			Average ash content \pm SD	SNI standard
	1	2	3		
FI	1.19	1.13	1.17	1.16 \pm 0.03	< 1.5
FII	1.10	1.15	1.18	1.14 \pm 0.04	
FIII	1.07	1.13	1.14	1.11 \pm 0.03	

Table 6. The Result of Polyphenol Qualitative Test on Instant Powder

Sample	Results	Interpretation
FI	Dark brown	+
FII	Dark brown	+
FIII	Dark brown	+

Table 7. The Result of Polyphenol Contents on Instant Powder

Sample	Replication			Mean levels \pm SD (mg GAE/g)
	1	2	3	
FI	45.60	43.76	42.13	43.83 \pm 1.73
FII	35.09	33.66	34.47	34.40 \pm 0.71
FIII	28.76	30.29	29.47	29.50 \pm 0.76

Table 8. Instant Powder Antioxidant Activity Test Results

Sample	IC ₅₀ value (ppm)	Antioxidant properties
Gallic acid standard	23.67	Very strong
FI	119.25	Moderate
FII	129.25	Moderate
FIII	146.40	Moderate

Based on table 8, IC₅₀ value of the gallic acid reference standard was 23.67 ppm. It was considered as very strong antioxidant category, while IC₅₀ value of formulation I, II, and III were considered as moderate antioxidant category.

DISCUSSION

Instant powder preparations of katuk leaves and cocoa beans were evaluated for physical quality to determine the feasibility and quality value of the product in accordance with the quality standard parameters that have been set for instant powder preparations. Physical quality tests carried out on this preparation included organoleptic, moisture content, and ash content. Organoleptic tests were carried out to see the acceptability of a product in terms of color, smell, and taste ¹. Based on the research, it was found that the instant powder preparations had a slightly sweet taste with the colors of the F1, F2, and F3 were dark brown, brown, and slightly yellowish brown, respectively. The aroma in formulation I has a characteristic smell of cocoa, while formulations II and III have a specific smell of katuk leaves. The results of this study have met the quality requirements of instant powder according to SNI 19-0428-1996 which are normal smell, taste, and color typical of spices. Based on the research, it was found that the moisture content of instant powder combined with katuk leaves and cocoa beans in formulation I was $1.71 \pm 0.04\%$, formulation II was $1.68 \pm 0.03\%$ and formulation III was $1.59 \pm 0.03\%$.

The results of this study found out that the more katuk leaf extract, the lower moisture contained in instant powder. According to research by Rusdiah *et al.* ³, the higher the katuk leaf extract, the lower the water content of the granules. This is reinforced by the statement of Hadi and Siratunisak ¹⁶ that the presence of additional ingredients such as cocoa powder in the manufacture of bran instant drinks can increase the value of the water content. Based on the research, it was found that the ash content of instant powder combined with katuk leaves and cocoa beans in formulation I was 1.16 ± 0.03 , formulation II was 1.14 ± 0.04 , and formulation III was 1.11 ± 0.03 . The results of this study indicate that the more content of cocoa bean extract, the higher the value of ash content. This is in line with research conducted by Hadi and Siratunnisak ¹⁶ that the more cocoa powder added to the bran instant drink, the higher the ash content value. The higher the value of the ash content, the more mineral contained in the material ¹⁷. Based on the results of this study, it has fulfilled the requirements of SNI 19-0428-1996 that instant powder may has ash content with no more than 1.5%. Furthermore, instant powder preparations were subjected to polyphenol qualitative tests, determination of polyphenol levels and antioxidant activity tests. Polyphenol qualitative test was carried out as a preliminary step to prove the presence of polyphenol content in instant powder preparation samples.

Based on the research results as stated in Table 6, the qualitative test results for instant powder polyphenols found that in formulation I, formulation II, and formulation III showed a blackish brown which indicated polyphenolic presence. These results are in accordance with previous research conducted by Sulasmi ¹⁸ that a sample is concluded to contain polyphenols if the color changes to black-brown. Based on the research, it was found that the polyphenol content of instant katuk leaf powder and cocoa beans in formulation I was 43.83 ± 1.76 mg GAE/g, formulation II was 34.40 ± 0.71 mg GAE/g, and formulation III was 29.50 ± 0.76 mg GAE/g. The levels of polyphenolic compounds are shown in GAE (Gallic Acid Equivalent) because the chemical structure of polyphenolic compounds in the instant powder of katuk leaves and cocoa beans is unknown ¹⁴. The results of this study indicate that formulation I with the most cocoa bean extract composition has the highest polyphenol content value. According to Towaha's research (2014) the polyphenol content in cocoa bean extract was 82.3 mg GAE/g. Meanwhile, according to Wongklom and Moonsin's

research ⁶, katuk leaves contain lower polyphenolic compounds, with value of 19.40 mg GAE/g. This explains that formulation I with the most cocoa bean extract composition, showed highest levels of polyphenols. There was a decrease in the levels of polyphenols between before and after being made into the preparation because it had gone through a cooking process by heating. Previous research reported that polyphenol group compounds are compounds that are thermolabile or easily damaged by temperature ².

Based on the antioxidant activity test, the results of the standard linear regression equation for gallic acid comparator were $y = 0.4573x + 39.175$ with a value of $R^2 = 0.9979$. From the linear regression equation, the IC_{50} value then was determined. From the calculation results, the standard IC_{50} value for gallic acid as a comparator was 23.67 ppm which was classified as a very strong antioxidant. Furthermore, in testing the antioxidant activity of samples of instant katuk leaf powder and cocoa beans, the results of the linear regression equation were obtained for the formulation I $y = 0.1599x + 30.931$ with $R^2 = 0.9985$; formulation II $y = 0.1513x + 30.443$ with $R^2 = 0.9961$, and formulation III $y = 0.1441x + 28.903$ with $R^2 = 0.9968$. These equations were used to determine the IC_{50} value of the samples. From the calculation results, the IC_{50} value of formulation I was 119.25 ppm; formulation II was 129.25 ppm, and formulation III was 146.40 ppm. These values indicated moderate antioxidant activity category.

In this study, the best antioxidant activity results were found in formulation I with the the highest cocoa bean extract composition compared to katuk leaf extract. According to research by Ghozaly and Herdiyanti ¹⁵, the ethanol extract of katuk leaves was included in the category of moderate antioxidant activity. Meanwhile, according to research by Diantika *et al.* ¹⁸, the ethanol extract of cocoa beans has antioxidant activity in the strong category. These studies may explains the highest antioxidant activity of formulation I compared to formulation II and III.

CONCLUSION

The organoleptic test results showed that the instant powder had a slightly sweet taste with the colors of the three formulations successively dark brown, brown and slightly yellowish brown. The aroma in formulation I has a characteristic odor of cocoa, in formulations II and III it has a distinctive odor of katuk leaves. The results of the instant powder moisture content test were $1.71 \pm 0.04\%$, $1.68 \pm 0.03\%$, and $1.59 \pm 0.03\%$. The results of the instant powder ash content test were $1.16 \pm 0.03\%$, $1.18 \pm 0.04\%$, and $1.11 \pm 0.03\%$. The polyphenols contents in instant powder with F1, F2, and F3 were 43.83 ± 1.76 mg GAE/g, 34.40 ± 0.71 mg GAE/g, and 29.50 ± 0.76 mg GAE/g, respectively. The instant powder has medium antioxidant category with an IC_{50} value were 119.25 ppm, 129.25 ppm, and 146.40 ppm for F1, F2, and F3.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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