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Exploration of Instant Functional Drinks Based on *Angkak* and Kidney Bean Flour: Implications on Bioactive Compound and Antioxidant Activity

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ABSTRACT

Indonesian eating patterns have shifted to more fast food, which is high in fat and free radicals. Free radicals are unstable and highly reactive molecules and will react with surrounding molecules to obtain electron pairs. Angkak and kidney bean flour contain bioactive compounds such as alkaloids, phenols, terpenoids, triterpenoids, flavonoids, isoflavonoids, flobatananis, coumarins, and saponins that could act as antioxidants. Both food ingredients can be modified into functional instant drinks to increase their benefits in providing daily antioxidant intake. Instant powder drinks are one of the alternative ways to get the benefits of plants or food. They have a pharmacological effect health, as they are used or consumed more easily and practically. This study aimed to contribute to producing an instant functional drink high in bioactive compounds and antioxidants as an alternative functional food. The research design used was a single-factor Complete Randomized Design (CRD) with four treatments, each repeated 3 times, so that 12 experimental units were obtained. The treatment in the study of making instant powder drinks consisting of the ratio of angkak and kidney bean flour was P1 (50:50%), P2 (40:60%), P3 (30:70%), and P4 (20:80%). Phenol, flavonoid, and antioxidant activity values were highest successively in the F4 treatment of 31.895 mg/L, 47.800 mg/L, and 60.996 µg/ml. The results show that instant powder drinks of kidney bean flour and angkak are included in the strong category and can be used as functional drinks high in antioxidants.

KEYWORDS

Antioxidant; *Angkak*; Functional drinks; Instant powder drink; Kidney bean flour

1. INTRODUCTION

In the current era of globalization, the consumption patterns of Indonesians have generally changed from consuming natural foods to fast food rich in fats and free radicals [1]. Free radicals are unstable and highly reactive molecules because they contain one or more unpaired electrons on their outermost orbitals. Free radicals react with surrounding molecules to achieve stability and obtain electron pairs [2].

Efforts to prevent diabetes mellitus, narrowing of blood vessels, coronary heart disease, stroke, and cancer can be done by regulating the consumption of nutrients that enter the human body, one of which is by consuming functional food products in the form of foods or drinks that contain antioxidants [3]. Functional food is a group of foods or drinks that contain ingredients estimated to improve health status or can prevent certain diseases, one of which is dosage formulations of instant powder drinks. One of the functional food products is in the form of instant powder drinks [2].

Instant powder drinks are one of the alternative ways to get the benefits of plants or food. They have a pharmacological effect on health, as they are used or consumed more easily and practically. Instant powder drinks, according to Indonesian Standard for Traditional Powder Drink SNI 01-4320-1996 [4], are products in the form of powders or granules made from a mixture of sugar and spices with or without the addition of other foodstuffs and permitted food additives.

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Efforts to make instant health drinks continue to be carried out, among others, by various formulations of natural ingredients that contain high amounts of antioxidants. *Angkak* and kidney bean flour can be used as instant powder drinks to support the availability of antioxidants. This is because the numbers contain alkaloids, phenols, terpenoids, triterpenoids, flavonoids, isoflavonoids, flobatananis, coumarins, and saponins, which are antioxidants. Although *angkak* has advantages compared to kidney beans, it has a lower zinc, protein and fibre content than kidney beans. The substitution of kidney beans in the manufacture of instant powder drinks can increase the content of antioxidants, proteins, fats, and fibre, as well as the acceptability of the product [5].

This research is innovative in formulating kidney bean flour with *angkak* as instant powder drinks. This is the first instant beverage formulated from *angkak* (red yeast rice) and red kidney bean. The instant powder drinks are estimated to have high phenolic content, high flavonoid content, and high antioxidant activity, so that they can be used as food or functional drinks rich in antioxidants.

2. MATERIALS AND METHODS

2.1. Materials

The samples used were kidney beans, *angkak* and granulated sugar sourced from a Supermarket in Samarinda City. Citric acid comes from Anggana Catur Prima Ltd (Jakarta, Indonesia), and the chemicals used in this study are analytical grade (Merck, Germany).

2.2. Kidney Bean Flour Production

The production of kidney bean flour begins with washing kidney beans that have been sorted from physical contaminants in running water, then soaking them in a 4.2% $NaHCO_3$ solution (w/v) for 150 minutes [6]. The kidney beans were cooked for 135 seconds at 121 °C using a pressure cooker at 15 psi. After water was drained, the cooked kidney beans were dried for 18 hours in a cabinet dryer at 60 °C, then ground with a grinder, and the flour was sifted with a sieve of 60 mesh.

2.2. Functional Instant Drinks Production

The manufacture of this formula was started with weigh the *angkak* and kidney bean flour, which were weighed separately according to the formulation determined in Table 1. Each ingredient was added to water according to the weight of *angkak* and kidney bean flour, respectively (e.g, for 16 g *angkak* added with 16 mL water). Each solution was filtered using a clean cloth until the juice of *angkak* and kidney bean flour were obtained. The juice that has been obtained was then decanted for 10 minutes, and then boiled in a pan at 43–44 °C. The sugar was added while stirring using a wooden spoon until crystalline grains were formed. The crystalline powder that has been formed is then cooled at room temperature for about 15 minutes and ground until it becomes a fine powder. The fine powder was then sifted using an 80-mesh sieve. The functional drink powder obtained was brewed using 200 mL of warm water at 60 °C [7].

Table 1. The formulation of an instant functional drink from kidney bean flour and *angkak*.

Ingredients	F1 (50%:50%)	F2 (40%:60%)	F3 (30%:70%)	F4 (20%:80%)
Angkak (g)	10	12	14	16
Kidney bean flour (g)	10	8	6	4
Water (mL)	20	20	20	20
Sugar (g)	40	40	40	40

2.3. Total Phenol Measurement

The total phenol content was measured using the Folin-Ciocalteau solution, with the comparison solution being gallic acid [8]. This measurement was done in triplicate. A total of 10 mL of infusion and 10 mg of Freeze-dried Infusa (FDI) *Plocoglottis lowii* Rchb.f. were used as the standard curve, and then dissolved in 10 mL of aquadest were used as samples. A total of 1 mL of sample solution was taken, and 2 mL of Na_2CO_3 was added. A total of 0.5 mL of Folin-Ciocalteu reagent was dissolved in as much as 5 mL and incubated for 5 minutes at 50 °C, then measured by UV/Vis spectrophotometer at λ 757 nm. The total phenol value is expressed in % w/w Gallic Acid Equivalent (GAE).

2.4. Total Flavonoid Measurement

Total flavonoid measurement was done in triplicate using as much as 25 mL of infusion and 25 mg of FDI *Plocoglottis lowii* Rchb.f. Comparators used in this research were a compound quartz and a routine use method [8]. A 1 mL of sample solution then added with 0.1 mL of $AlCl_3$ 10% and 0.1 ml of 1 M Na-acetate then incubated for 30 minutes and measured by UV/Vis spectrophotometer at λ_{max} 434 nm for gallic acid compounds and λ_{max} 509.5 nm for the compound routinely, the total flavonoid value is stated in % w/w Quarcetin Equivalent (QE) and Routine Equivalent (RE).

2.5. Antioxidant Activity Measurement

The DPPH stock solution of 125 µM was prepared by dissolving as much as 2.5 mg DPPH into ethanol p.a in the measuring flask and bringing it to a volume of 50 mL. Then the measuring flask was coated with aluminium foil [8]. The standard vitamin C and quercetin were weighed at 10 mg each. Then, it is dissolved with as much as 1 mL of aquabides, sonicated until dissolved, and then vortexed. Standard solutions are made into several concentrations (0.625, 1.25, 2.5, 5, and 10 ppm). Samples were made into multiple concentrations (150, 500, 1500, 5000, 15000, and 50000 ppm), which were taken based on considerations to meet all concentration ranges of the concentration of a single serving sample after being calculated using blood dilution calculations. As a supernatant of the stock solution, the sample was centrifuged at the preparation stage and put into a microplate with 100 µL. Furthermore, 100 µL DPPH was added to the sample, while for the negative control, only 100 µL aquabides was added to the sample. After that, incubation was carried out at room temperature in the dark for 30 minutes. The color intensity readings were carried out using a spectrophotometer at a wavelength of 517 nm. The blank solution used was 100 µL of aquabides, and 100 µL of DPPH was added, while for the negative control, 200 µL of aquabides was used. The determination of free radical inhibition capacity (%) used equation (1).

$$Free\ Radical\ Inhibition\ Capacity\ (\%) = \frac{A - B}{A} \times 100 \tag{1}$$

Where A is the absorbance of the blank solution (DPPH and ethanol) and B is the absorbance of the sample (DPPH, ethanol, and sample). The absorbance value of the sample is reduced by the correction absorbance, which is the absorbance value of the sample only. The inhibition percentage value is plotted into a curve as the y variable and the sample concentration as the x variable. The line equation is carried out by determining the line equation used based on the highest R^2 value. The capacity value is expressed in the percentage of IC_{50} (the effective concentration of the sample capable of inhibiting 50% of DPPH radicals). The IC_{50} value is calculated based on the line equation obtained by entering the value of 50 as the y variable and looking for the value of the x variable as the concentration of the sample sought.

3. RESULTS AND DISCUSSION

Bioactive compounds such as flavonoids are contained in ingredients such as kidney bean and *angkak* (red yeast rice). A recapitulation of the analysis of chemical characteristics and post-hoc test of the various influences of numerical formulations and kidney bean flour on the antioxidant activity of instant powder drinks is presented in Table 2. The recapitulation of the post hoc test results shows that numerical substitution has a very noticeable effect on total phenols, total flavonoids, and antioxidant activity.

3.1. Total Phenol

Table 2 shows the average total phenol from the instant powder drink of kidney bean flour formulation, with 10.49 to 33.473 mg/L. The highest total phenol is found in the F4 with a total phenol of 33.473 ± 0.525 mg/L, while the lowest is F1 with a total phenol of 10.493 ± 0.804 mg/L. The higher the percentage of *angkak* in instant powder drinks, the higher the total phenols showed in analysis. This is thought to be because *angkak* contains antioxidant compounds classified as polyphenols. *Angkak* contains phenolic acid of 1.89 mg GAE/g extract [9].

Antioxidants in numerals consist of several compounds such as polyphenols, flavonoids, alkaloids, carotenoids and vitamins [10]. Some of the metabolite compounds produced by the fungus *Monascus* are composed of polyketides. These components are pigments and phenolic compounds with high antioxidant activity [11]. *Angkak* contains several antioxidants such as dimerumic acid, tannins, and phenols [9]. Meanwhile, red kidney bean flour contains several bioactive compounds such as catechol 17.63 µg/mL, chlorogenic 6.12 µg/mL, syringenic 5.41 µg/mL, coumaric 10.60 µg/mL, caffeic 14.55 µg/mL, pyrogallol 13.12 µg/mL, and protocatechulic 6.33 µg/mL [12].

Table 2. Total phenol, total flavonoid, and antioxidant activity of the formulation of the drink instant powder of kidney bean flour and *angkak*.

Formulation (Kidney bean flour:*Angkak*)	Total phenol (mg/L)	Total flavonoid (mg/L)	Antioxidant activity IC_{50} (µg/mL)
F1 (50%:50%)	$10.493^a \pm 0.804$	$34.269^a \pm 0.226$	$71.392^c \pm 1.125$
F2 (40%:60%)	$11.893^b \pm 0.525$	$36.245^b \pm 1.018$	$72.153^c \pm 1.916$
F3 (30%:70%)	$16.046^c \pm 0.617$	$37.874^b \pm 1.682$	$63.467^b \pm 0.954$
F4 (20%:80%)	$33.473^d \pm 0.525$	$47.800^c \pm 0.667$	$60.330^a \pm 0.945$

Description: The different letters behind the numbers show a noticeable difference in significance level α 5%.

3.2. Total Flavanoid

The total flavonoids produced from instant powder drinks formulation of kidney bean flour and the highest numerals were 47.800 mg/L in the F4 treatment (20% kidney bean flour:80% *angkak*) and the lowest flavonoid content value in the F1 treatment (50% kidney bean flour:50% *angkak*) with a total flavonoid value of 34.269 mg/L. The higher the addition of *angkak* powder, the higher the total flavonoid content of instant powder drinks. *Angkak* contain several flavonoids such as daidzein, genistein, 5,5'-Dimethoxylariciresinol, lariciresinol, and scopoletin [13].

Red kidney bean contains several flavonoids such as rutin 1.56 µg/mL, naringin 6.56 µg/mL, quercetin 9.12 µg/mL, kaempferol 4.24 µg/mL, hesperidin 11.37 µg/mL, and catechin 10.41 µg/mL [12]. Flavonoids are one of the largest groups of phenol compounds found in nature. These compounds are red, purple, and blue dyes, and some yellow dyes in plants [14]. Flavonoids are polar compounds because they have several unsubstituted hydroxyl groups [15]. This flavonoid compound can be an anti-microbial, wound infection drug, anti-fungal, anti-viral, anti-cancer, and anti-tumor [16]. In addition, flavonoids can also be used as anti-bacterial, anti-allergic, cytotoxic, and anti-hypertensive [17]. Flavonoids act as antioxidants by utilizing their redox properties, namely as reducing agents by capturing free radicals through hydrogen atom donors from the flavonoid hydroxyl group and stabilizing singlet oxygen, which causes radicals to become inactive so that they can inhibit lipid oxidation [18], [19].

3.3. Antioxidant Activity

Furthermore, the highest antioxidant activity value produced from instant powder drinks formulation of kidney bean flour and the highest numerals was 60.330 µg/mL in the F4 treatment (20% kidney bean flour:80% *angkak*) and the lowest antioxidant activity in the F1 treatment (40% kidney bean flour:60% *angkak*) with antioxidant activity of 72.153 µg/mL. The antioxidant activity in instant powder drinks in the F4 treatment belongs to the category with vigorous antioxidant activity, with a relatively small average IC_{50} value of 60.330 µg/mL and IC_{50} values ranging from 50-100 µg/mL have a strong antioxidant intensity.

Based on the data above, the higher the percentage of *angkak* (red yeast rice), the higher the antioxidant activity of the instant powder drink produced. This is due to bioactive compounds such as polyphenols, carotenoids, alkaloids, and several other types [20], [21]. Red yeast rice or *angkak* also contains rubropunctamine, rubropunctatin, monascorubramine, monascin, and other pigments [22]. Other compounds present are terpenoids, triterpenoids, flavonoids, flobatananis, coumarins and saponins. Phenols are important compounds that act as antioxidants. Phenolic acid will bind to hydroxyl, phenoxyl and superoxide. In addition, phenolic acid can also increase the effectiveness of antioxidant enzymes and the production of antioxidant proteins in the body. The amount of phenolic acid in the numerical figure is 1.89 mg GAE/g extract. Meanwhile, the vitamins contained in numerals are ascorbic acid as much as 3.09 μg GAE/g extract and contain other compounds such as lovastatin, flavonoids, terpenoids, triterpenoids, phenols, and saponins [9], [23]. Table 2 shows that a higher percentage of *angkak* showed higher phenolic content and higher flavonoid content. This result is similar to the previous study of chocolate beverages, which showed higher phenolic than higher flavonoid and higher antioxidant activity [24].

Antioxidants are compounds that can slow down and prevent oxidation processes caused by free radicals [25], [26]. Antioxidant activity is expressed as the percentage of inhibition obtained from the absorbance value of blanks reduced by sample absorption [26], [27], [28]. Methods for determining antioxidant activity have been widely used to determine the antioxidant activity of antioxidant-containing ingredients. One of the methods of determining antioxidant activity that is popularly used is the DPPH method. The DPPH method is simple, inexpensive, and fast for measuring antioxidant activity [29]. The DPPH method uses the free radical compound 1,1-diphenyl-2-picrylhydrazyl (DPPH) as the target to be counteracted by the sample. This method can be utilized for solid and liquid samples and is not specific to certain antioxidant compounds [30].

4. CONCLUSION

The highest total phenol and flavonoids were found in F4, with the concentration of 33.473 mg/L and 47.800 mg/mL, respectively. The highest antioxidant activity value was produced from F4 with an IC_{50} of 60.330 μg/mL, although all the formulations showed strong antioxidant activity. The best formulation for an instant functional drink was F4, based on measuring phenolic, flavonoid content and its antioxidant activity.

AUTHOR CONTRIBUTION

All authors contributed equally to the main contributors to this paper. All authors read and approved the final paper. **Rinten Anjang Sari**: Writing (review & editing), writing (original draft), formal analysis, and funding acquisition. **Riana Pangestu Utami**: Writing (review & editing), writing (original draft), investigation, and formal analysis. **Umboh Ruth Gloria Natalie**: Investigation, writing (review & editing).

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