

FORMULATION DEVELOPMENT AND MEASUREMENT OF ANTIOXIDANT ACTIVITY OF *ELEUTHERINE AMERICANA* MERR TEA CUBE

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ABSTRACT

Objective: The current study was conducted to develop *Eleutherine americana* Merr (Bawang dayak) as Teacube and to investigate its antioxidant activity.

Methods: *Eleutherine americana* Merr was extracted using the infusion method. To make the tea cube, this extract was combined with molasses and granulated sugar. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition method was used to assess antioxidant activity. Finally, a hedonic test was used to determine the formula's acceptability.

Results: The antioxidant activity in the *Eleutherine americana* Merr teacube formula was found to have IC₅₀ values of 60.35, 51.87, 79.85, and 53.36 ppm for formulas I,II,III,IV, respectively. The result of hedonic test showed that formula II had the best acceptance for color (3.53), flavor (3.58), odor (3.67), texture (4.00) and acceptability (4.00)

Conclusion: *Eleutherine americana* Merr teacube formulation presented has antioxidant activity and has great for antioxidant agent and also can be accepted by the panelists

Keywords: Bawang dayak, Antioxidant, Tea cube, and *Eleutherine americana* merr

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INTRODUCTION

The potential of forests and plants is still widely used by the community as part of their lives in Kalimantan, and *Eleutherine americana* Merr or locally known as bawang dayak are one of the plants that are widely used as alternative medicine by the people of Kalimantan [1]. *Eleutherine americana* Merr have been shown to be effective in the treatment of a variety of diseases, including wounds, coughs, and stomachaches [2]. Traditional medicine is becoming more popular as a means of dealing with health issues. This is due to the fact that it is thought to be safer than synthetic drugs [3].

Eleutherine americana Merr is a plant native to East Kalimantan. This plant in the *Iridaceae* family that belongs to the genus *Eleutherine* and the species *Eleutherine palmifolia* Merr [4]. *Eleutherine americana* Merr have a shape similar to shallots, but they are slightly larger in size and have thicker layers than shallots [5].

Eleutherine americana Merr bulbs have traditionally been used as a medicine for breast cancer, while the leaves aid in the production of breast milk [6]. The Dayak community has used this plant for generations as a medicinal plant, especially as a medicine for various diseases such as breast cancer, high blood pressure (hypertension), diabetes mellitus, cholesterol-lowering medicine, ulcers, colon medicine, cancer and stroke prevention [7, 8].

Furthermore, *Eleutherine americana* Merr contains antioxidant components that help to protect the body's health. This activity is related to phytochemicals, including alkaloids, glycosides, flavonoids, phenolic, tannins, and steroids [9]. Antioxidants are substances that can either delay or prevent free radical oxidation reactions. Free radical can be affected in cellular component such as DNA and RNA, which leads to damage [10, 11]. Free radical also has been linked to the development of lifestyle-related disorders such as atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and cancer [12].

A plant's antioxidant activity has the potential to be developed as a health-improving ingredient or as a nutraceutical [10]. A nutraceutical is a food (or a component of food) that offers medical or health advantages, such as illness prevention and/or treatment [13].

People nowadays prefer to use products whose packaging and presentation are more practical and quick, as they do not require a lot of time to prepare. People's lifestyle choices have also increased the demand for healthy products that provide health benefits, particularly disease prevention [14]. Tea is a popular product among the general public today. Tea drinks are popular due to their refreshing taste and soothing aroma [15].

Eleutherine americana Merr are widely used to make tea drinks. The most popular *Eleutherine americana* Merr tea products on the market today are tea bags and brewed simplicia. These products have flaws, including an unappealing appearance and the requirement to use hot water in their presentation. As a result, it is necessary to create new, more user-friendly products. The tea cube is a tea-making innovation that is simple, appealing, stable, and easy to serve without the use of hot water. This product can also preserve the authenticity of the tea's flavor and stability. Tea cubes can also reduce the use of tea bags, making them very environmentally friendly.

Based on the foregoing, this study intends to create a nutraceutical product of *Eleutherine americana* Merr tea cube as a herbal superfood drink. The antioxidant activity and acceptability of the *Eleutherine americana* Merr tea cube formula will also be determined in this study.

MATERIALS AND METHODS

Material

The main ingredients that used in this research are *Eleutherine Americana* Merr the sample was collected from the Tenggara Seberang (L3) area of East Kalimantan. Sample the identified at the Mulawarman Herbarium, Laboratory Ecology and Conservation Tropical Forest Biodiversity, Forestry Faculty, University of Mulawarman, Samarinda, East Borneo, with the identity number 104/UNI7.4.08/11/2023. Granulate sugar, brown sugar, distilled water, methanol (Merck), DPPH (Merck), glassware (Iwaki), Spectrophotometer UV-Vis (Thermo Scientific), micropipette (Dragon lab).

Preparation of teacube formula

Eleutherine americana Merr granules are made using four different formulas, each with a different amount of sugar as shown in table 1. Tea cubes are produced in four stage: preparation infusion, preparation of granule, preparation of molasses, and tea cube formulation.

Preparation of *eleutherine americana* Merr infusion

Eleutherine americana Merr are cleaned of dirt and washed once with running water. The samples were then cut into pieces and dried for 48 h in indirect sunlight. After that, the sample was extracted using the infusion technique [13]. Ninety (90) g of sample were weighed and placed in an infusion pan, followed by 432 ml of distilled water and 15 min of boiling at 90 °C. Following that, the infusion results were filtered.

Preparation of *eleutherine americana* Merr granule

One hundred (100) ml of *Eleutherine americana* Merr infusion is mixed with different amount of sugar (120-480 g) and stirred until homogeneous. The mixture is then boiled over low heat, stirring until crystalline powder forms are form.

Preparation of molasses

Two hundred and forty (240) g granulated sugar and 120 g of brown sugar were mixed in pan. The mixture is then slowly heated until molasses is obtained.

Tea cube formulation

Four tea cube formulation of *Eleutherine americana* Merr, namely F1, F2, F3 and F4 were mixed into different proportions, especially in granulated sugar as shown in table 1. The mixture is thoroughly mixed before being molded in a silicon mold.

Table 1: Formulation of tea cube *Eleutherine americana* merr

Formulation steps	Material	Formula			
		I	II	III	IV
<i>Eleutherine americana</i> Merr	<i>Eleutherine americana</i> Merr (g)	90	90	90	90
Infusion	Distilled water (ml)	432	432	432	432
<i>Eleutherine americana</i> Merr	<i>Eleutherine americana</i> Merr Infusion (ml)	100	100	100	100
Granule	Granulated Sugar (g)	120	240	360	480
Mollases	Granulated Sugar (g)	240	240	240	240
	Brown Sugar (g)	120	120	120	120
Formulation of tea cube	<i>Eleutherine americana</i> Merr Granule (g)	240	240	240	240
	Mollases (ml)	50	50	50	50

(All quantities are in g/ml)

Determination of antioxidant activity of *eleutherine americana* merr infusion and tea cube of *eleutherine americana* merr using DPPH method

Determination of antioxidant activity on *Eleutherine americana* Merr infusion and *Eleutherine americana* Merr tea cube were made by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method using UV-Vis spectrophotometry at 517 nm. Ascorbic acid (1 mg/ml) was used as a positive control. Ascorbic acid (positive control) was prepared at a concentration range of 3.125 to 12.5 g/ml, and samples containing *Eleutherine americana* Merr infusion and *Eleutherine americana* Merr k tea cube were prepared at a concentration range of 5 to 300 µl/ml. Ascorbic acid and samples were pipetted, and then 3 ml of DPPH solution and 96% methanol were added to 10 ml in a volumetric flask. Samples were then incubated for 30 min in the dark for further absorbance readings using UV-Vis spectrophotometry at 517 nm and 96% methanol as a blank. Samples are used to determine percent inhibition. Each sample was tested three times [16].

Hedonic test

The hedonic test is a test of a person's level of preference for a product consumed, so it is also called a sensory test [17]. In carrying out a hedonic test, panelists gave an assessment of the level of preference based on observations using the five senses. Panelists were asked to rate how much they liked the color, taste, and aroma of the cube tea product, the scale given was as follows: 1. Really like it, 2. Like it, 3. Neutral, 4. Dislike.

Data analysis

The hedonic test data were statistically examined, and the findings were summarized with Analysis of Variance (ANOVA) on the data. The threshold of significance between various parameters was assessed at 5% ($P < 0.05$). Duncan's follow-up test was included in the analysis, which was performed using the SPSS statistic 24 tool.

RESULTS AND DISCUSSION

Eleutherine americana Merr are beneficial plants that are traditionally consumed as brewed tea by the people of Kalimantan [18]. *Eleutherine americana* Merr tea cubes formulation aims to

simplify the stages of tea preparation so that users feel more at ease serving it. This goal will then increase consumption of beneficial drinks, which will increase nutritional intake [19].

The main difference between the four formulas for *Eleutherine americana* Merr is the addition of granulated sugar when making *Eleutherine americana* Merr granules. This is to examine the differences in sweetener addition and their relationship with taste acceptability in acceptance testing and also in the appearance of making the tea cube. There was no discernible difference in the appearance of all tea cube formulas (fig. 1). Tea cubes are square in shape, brown in color, and have a sweet aroma. Sugar is a main ingredient in tea consumption also in tea cube formulation. Sugar as one of the main components in tea consumption, the purpose of adding sugar is to form a consistency of the preparation so that it becomes firm and also functions as a natural preservative. *Eleutherine americana* Merr have a slightly bitter and spicy taste, adding sugar can cover the sour taste of *Eleutherine americana* Merr, and *Eleutherine americana* Merr do not have a distinctive aroma like onions in general.

Determination of antioxidant activity of *eleutherine americana* Merr infusion and *eleutherine americana* Merr tea cube

The antioxidant activity of *Eleutherine americana* Merr infusion and *Eleutherine americana* Merr tea cube samples was measured using the DPPH method. The antioxidant value of the *Eleutherine americana* Merr infusion samples was first determined, followed by comparison to the antioxidant results in the formula. This study seeks to determine whether the addition of formula ingredients affects the antioxidant activity of *Eleutherine americana* Merr infusion. The measurement results show that the IC₅₀ value of *Eleutherine americana* Merr infusion is 11.11 ppm, while ascorbic acid as a positive control is 3.77 ppm; both of these values fall into the strong antioxidant category [20, 21] Meanwhile, the antioxidant results in formulas I-IV show an antioxidant value ranging from 51.87 to 79.85 ppm, which is considered a strong antioxidant (fig. 2). The results revealed that when *Eleutherine americana* Merr infusion was formulated in tea cube form, its antioxidant activity decreased. However, this decrease was not significant because *Eleutherine americana* Merr tea cubes still exhibited strong antioxidant activity. Furthermore, adding sugar to tea does not significantly reduce its antioxidant effect but rather stabilizes it [22, 23].



Fig. 1: The appearance of *Eleutherine americana* Merr tea cube

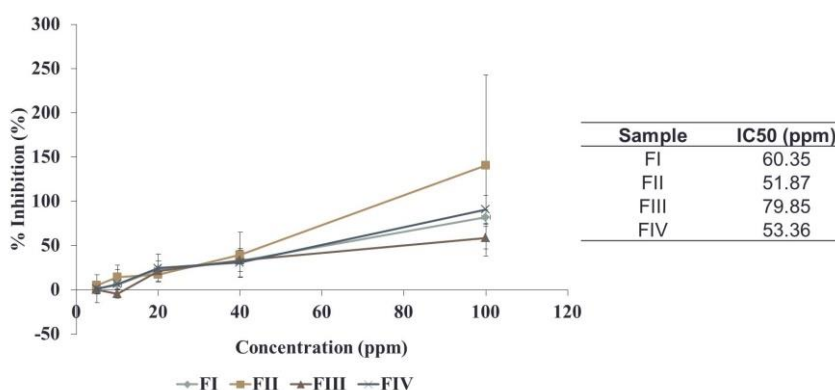


Fig. 2: Antioxidant activity of tea cube formulation of *Eleutherine americana* Merr (FI-IV)

Hedonic test

Hedonic test are essential to monitor quality of new develop product and to make sure that product were acceptable at consumer level. The developed *Eleutherine americana* Merr tea cube formulation were evaluated on the five parameters that is odor, color, flavor,

texture and acceptability. According to the (table 2). All the formula has not significantly different ($P < 0.05$). Among the *Eleutherine americana* Merr tecube formula, Formula II had the most preferred for all parameter with the highest score. Color, odor and flavor is an important parameter for food industry it can be stimulate appetite and product selection [24, 25].

Table 2: The result of hedonic test on *Eleutherine americana* merr tea cube formulation

Hedonic Aspects	Formula			
	I	II	III	IV
Color	3.27±0.46	3.53±0.74	2.67±0.49	2.53±0.53
Flavor	3.20±0.41	3.85±0.21	2.80±0.41	3.13±0.74
Odor	3.13±0.35	3.67±0.82	3.07±0.80	3.00±0.65
Texture	3.27±0.46	4.00±0.76	3.75±0.09	3.73±0.21
Acceptability	3.27±0.46	4.00±0.76	3.27±0.59	3.27±0.46

*mean values with the column with same superscript do not differ significantly ($p < 0,0$)

CONCLUSION

The results of sensory analysis using the hedonic test method on aroma, texture, color, taste and acceptability are formula II has the highest score and this is comparable to antioxidant activity with the IC50 5.67 for the formula II

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AUTHORS CONTRIBUTIONS

Ika A. Mentari and Indah Hairunisa and Eva Mardiana designed the study, collected samples, extracted them, tested them for tea cube formulation and antioxidant activity, and wrote the manuscript Paula M. Kustiawan, Mohd F. A. Bakar, Fazleen I. A. Bakar designed the study, assisted with data analysis, and wrote the manuscripts.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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