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Phytochemical Screening of Bangun-Bangun Leaves Extract (Plectranthus Amboinicus) and its Formulation in Biscuits

Indri S Siregar[1], Hotnida Sinaga[2,*], Jansen Silalahi[3]

[1]Department of Food Science, Faculty of Agriculture, Universitas Sumatera Utara
[2]Department of Food Science, Faculty of Agriculture, Universitas Sumatera Utara
[3]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara

Abstract – The drying of the leaves was carried out using an oven with 2 temperature variants, namely 60°C and 80°C, and 3-time variants, namely 90, 120, and 150 minutes. Antioxidant activity analysis was carried out using the DPPH method and qualitative phytochemical analysis: Alkaloids, Flavonoids, Steroids/Triterpenoids, Saponins. The basic of ingredients Biscuit are : butter, sugar, eggs, and wheat flour then performed a proximate analysis on biscuits. The results showed that the activity of the leaf extract was strong/moderate 63.53 g/ml in the leaf extract at 60°C and 90 minutes, drying at this temperature was used to make biscuits. The Extract of Bangun-bangun leaves with positive temperature and time variants contained flavonoid compounds and negative for steroidal alkaloids, for saponins there were positive and negative results in the extract of Bangun-bangun leaves with a drying temperature of 60°C for 150 minutes and 80°C for 120 minutes. The proximate analysis obtained was 4.38% water content, 1.87% ash content, 8.12% fat content, 10.68% protein content, 74.94% carbohydrate content, and antioxidant activity of 199.96 g/ml in biscuits. with the addition of Bangun-bangun leaves powder.

Keywords – Bangun-Bangun Leaves, Antioxidants, Phytochemicals, Drying And Biscuits.

I. INTRODUCTION

Bangun-bangun leaf is Indonesian ethnobotany that has been used by the people of North Sumatra as a daily vegetable menu for generations, especially for mothers who hasve just given birth, which has been shown to increase the total volume of breast-milk [1]. The leaf of bangun-bangun have a fairly short shelf life as do vegetables in general. This is a consideration even though the consumption of leaves has a significant effect on increasing levels of several minerals such as iron, potassium, zinc, and magnesium and can result in a significant increase in baby weight [2]. Currently, many people do not know the shape of the leaves because in some areas they think these leaves are only wild plants. Processed products like this have a low shelf life, so innovation is needed to increase the shelf life of the product and increase its economic value. The drying temperature and time affect the antioxidant activity. The purpose of this study was to determine the characteristics, the drying process on the bioactive components, save storage space and extend the shelf life of the leaves and its formulations in biscuits. The results of the study [3] the antioxidant activity(IC_{50}) of teki leaves at a temperature of 30 °C were 980.46 µg/ml while at a temperature of 50 °C it was 1616.97 µg/ml. Analysis of antioxidant activity was carried out [4] with time variance, the highest antioxidant activity was obtained in soursop leaves with a drying time of 150 minutes, which was 76.06% and the lowest was 53.17% at 30 minutes of drying. According to [5] the antioxidant activity of phenolic compounds is largely determined by the chemical structure, number, and position of the hydroxyl and methyl groups on the ring. The more molecules that are substituted for hydroxyl groups, the stronger the ability to scavenge DPPH free radicals because of the greater ability to donate hydrogen. The aim of the study was to formulate bangun bangun leaf extract in biscuit.

Corresponding Author: Hotnida Sinaga (hotnida@usu.ac.id)

II. Materials and Methods

a. Materials

The materials used in this study were fresh leaves obtained from Karo district, sulfuric acid, 10% NaOH, 3% H3BO3, HCL solution, a diethyl ether solvent, 2 N hydrochloric acid, Mayer's reagent, Dragendorff's reagent, Bouchardat's reagent, n-hexane, anhydrous acetic acid, DPPH solution, ethanol, distilled water.

b. Method

This research was conducted with 2 variants of temperature and 3 different drying times. Testing the characteristics of the leaves using a factorial Completely Randomized Design (CRD) consisting of 2 treatment factors: temperature 60 ^{o}C (A1) and 90 ^{o}C (A2) and the drying times were 90 (B1), 120 (B2), and 150 (B3) minutes.

i. Phytochemical Screening

Phytochemical screening includes an examination of alkaloids, flavonoids, and steroids. The material used is the leaves of bangun-bangun that have been dried. then macerated using mineral water solvent in a ratio of 1:10 for 24 hours.

- Alkaloids: A total of 0.1 ml of the material (the extract of Bangun-bangun leaves) was dissolved with 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated for 2 minutes, cooled, and filtered, the filtrate was used for the alkaloid test (Ditjen POM, 1995).
- Flavonoids: A total of 0.1 ml of material (leaves extract) + 1 ml of NaOH ml → Positive (Harbone, 2008 in Taher, 2011). Positive flavonoids if there is a red or yellow or orange color on the amyl alcohol layer.
- Steroids : A total of 0.1 ml of material (leaves extract) + 2 ml of chloroform + 5 drops of H2SO4 6 M → Positive [6]. (Lieberman-Burchard reagent). If a blue-green color is formed, it indicates the presence of triterpenoids, while red, pink, or purple colors indicate the presence of steroids.
- Saponins: A total of 0.1 ml of the material (leaf extract) was put into a test tube, added 10 ml of hot distilled water, cooled, then shaken vigorously for 10 seconds. Saponins are positive if a stable foam is formed for not less than 10 minutes as high as 1 to 10 cm and with the addition of 1 drop of 2 N hydrochloric acids the foam does not disappear (Ditjen POM, 1995).

ii. Antioxidant Activity by DPPH Method

The sample of antioxidant activity test was determined by the DPPH method [9].

- Determination of DPPH Absorbance Curve: DPPH solution with a concentration of 40 ppm was homogenized and its absorption measured at a wavelength of 400-800 nm, then an absorption curve will be obtained where the Y-axis is the absorbance and the X-axis is the wavelength, and the wavelength where the maximum absorbance occurs [8]
- Determination of Percentage of DPPH Reduction: Calculation of antioxidant activity with DPPH reagent by calculating IC_{50} for each sample. The amount of % DPPH attenuation is calculated by:

  $$\text{Free radical scavenging activity (\%)} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \; x \; 100\%$$

  Notes: $A_{control}$ = Absorbance does not contain any samples

  A_{sample} = Sample absorbance
- Preparation of the Percent Attenuation Calibration Curve: This was done by making 5 series solutions of concentration levels from each sample (3.12; 6.25; 12.5; 25; 50, and 100 ppm) whose absorbance was found then plotted the concentration value to produce calibration curve. The attenuation obtained from absorbance measurements were measured at a maximum wavelength of 516 nm and with an incubation time of 30 minutes [9]. Then plotted percent attenuation and concentration to obtain a calibration curve, and regression equation Y = aX + b. IC 50 calculation was obtained from the linear regression equation where Y = aX + b,

  Y= % attenuation → X= concentration

IC 50 is calculated by entering the price Y = 50, so it can be calculated the concentration value of X

- Determination of Antioxidant Activity (IC_{50}): Test Solution of the value of antioxidant activity (IC_{50}) was obtained by first making a linear regression equation that connects the % reduction to the concentration of the test solution for each sample (3.12; 6.25; 12.5; 25, 50, and 100 ppm) and then a calibration curve was made and the regression equation calculated for each test solution. IC_{50} is obtained by calculating the concentration of the test solution that can produce free radical resistance (% attenuation) of 50 based on the linear regression equation using the formula: Y = ax + b

Description: Y = 50; x = test sample concentration (IC_{50}), then it will be obtained the value of x (IC_{50})

- Formulation Bangun-bangun Leaf in Biscuit

The process of making biscuits was carried out 2 times, namely: control biscuits and biscuits with the addition of Bangun-bangun leaves. The formulation of biscuit-making ingredients was a modification of Andriana (2017). The ingredients used were flour, butter, powdered sugar, baking powder, milk powder, and salt. The addition of Bangun-bangun leaf powder that is used is the leaf of Bangun-bangun leaf (A1B1). Baking biscuits that have been processed are baked at a temperature of 120ºC for 20-30 minutes.

iii. Antioxidant Activity of Biscuits with Bangun-bangun Leaf Powder Added

The biscuits that have been obtained are then weighed as much as 1 g and mashed and extracted using ethanol (pro analysis) then filtered, then pipetted 15.6; 31.2; 62.5; 125; 250 and 500 µg/ml to obtain a concentration of 3.12; 6.25; 12.5; 25; 50 and 100 ppm were then calibrated to 5 ml and then the antioxidant activity was determined spectrophotometrically.

III. Results and Discussion

a. Antioxidant Activity of Bangun-Bangun Leaf Extract

The results of the analysis of the antioxidant activity of the leaf extract can be seen in Table 1.

Table 1. Antioxidant Activity (IC_{50}) test sample:

Treatment	IC_{50} (µg/ml)	
A2B3	77,55 ± 1,44	A
A2B2	75,19 ± 2,38	A
A1B3	72,38 ± 3,98	a b
A1B2	69,11 ± 2,35	b c
A2B1	68,68 ± 1,28	b c
A1B1	63,53 ± 2,60	c

Note: Data is the average of 3 replicates

Inactive/weak antioxidant activity(IC_{50}) was found in A2B3 treatment with an average value of 77.55 µg/ml, while active/moderate antioxidant activity was produced by A1B1 treatment with an average value of 63.53 µg/ml. Based on the standard level of antioxidant activity, compounds that are categorized as very active/strong have IC_{50} values < 10 µg/ml, active/moderate categories if they have an IC_{50} value of 10-100 µg/ml, and IC_{50} values > 100 µg/ml are categorized as inactive/ weak [10]. Husni et al,[11] the higher the temperature and the longer drying time, the antioxidant activity will decrease. Antioxidant activity is expressed by the IC_{50} value. The IC_{50} value indicates the sample concentration required to inhibit 50% of free radical activity. Research conducted by Mulia et al,[12] the antioxidant activity of teki leaves at a temperature of 30 ^{o}C is 980.46 µg/ml while at a temperature of 50 ^{o}C 1616.97 µg/ml. Analysis of antioxidant activity was carried out by Delvi, and Wikanastri [13] with time variants. The highest antioxidant activity was found in soursop leaves with a drying time of 150 minutes, which was 76.06% and the lowest was 53.17% at 30 minutes of drying.

b. Phytochemical Screening of Bangun-bangun Leaf Extract

The results of the qualitative phytochemical test on the extracts of Bangun-bangun leaves can be seen in Table 2.

Table 2. Phytochemical Analysis of Bangun-Bangun Leaf Extract

Material	Chemical Content	Test Method	Result					
			60^{O}C			80 ^{O}C		
			90	120	150	90	120	150
Bangun-bangun leaf extract	Alkaloids	Mayer	-	-	-	-	-	-
		Dragendroff	-	-	-	-	-	-
		Bouchardat	-	-	-	-	-	-
	Flavonoids	Serbuk Mg + Amil Alkohol + HCl_p + HCl	+	+	+	+	+	+
	Steroids	Lieberman-Bouchat	-	-	-	-	-	-
	Saponins	Hot Water	+	+	-	+	-	+

Desc: - (no change with reaction), + (change occurs with the reaction)

Based on the results of qualitative testing that the extract of the Bangun-bangun leaves with temperature and time variants contained flavonoid compounds and negative for steroidal alkaloids, for saponins there were positive and negative results in the extract of Bangun-bangun leaf with a drying temperature of 60 ^{o}C for 150 minutes and 80 ^{o}C for 120 minutes. The variation of the test results obtained has a significant effect on the phytochemical content produced. This is due to changes during the drying process which are affected by temperature. The drying process in food can reduce the levels of flavonoids and antioxidant compounds. Biscuits

The results of the proximate analysis on biscuits without leaves and biscuits with the addition of leaves can be seen in Table 3.

Table 3. Biscuit Proximate Analysis

Sample	Water content (%) ± SD	Ash content (%) ± SD	Fat content (%) ± SD	Protein content (%) ± SD	Carbohydrate content (%) ± SD
Biscuits (Control)	4,69 ± 0,06	1,85 ± 0,01	9,00 ± 0,44	8,98 ± 0,26	75,44 ± 1,80
Bangun-bangun leaf Biscuits	4,38 ± 0,16	1,87 ± 0,09	8,12 ± 0,16	10,68 ± 0,22	74,94 ± 0,30

Note: data is the average of three replicates

The results of the analysis of the moisture content of the biscuits (control) were 4.69% and the Bangun-bangun leaf biscuits were 4.38%. The results of the analysis of moisture content are also influenced by the thickness of a food ingredient, in this study the biscuit molding process was carried out using a glass mold with a thickness of 0.5 mm so that the baking process could also be evenly distributed on the biscuits. Based on the Formula Quality Requirements according to SNI 2973:2011 which states the maximum water content is 5%, the biscuits (control) and Bangun-bangun leaf biscuits can meet these requirements.

Ash is inorganic substance leftover from the combustion of organic material. Ash content is related to the mineral content of a material. White ash is thought to be a mineral found in biscuits. Analysis of ash content in biscuits (control) was 1.85% and

Bangun-bangun leaf biscuits were 1.87%. According to De man [14], combustion carried out at a temperature of 550 ^{o}C will damage organic compounds and leave minerals in the samples tested for ash content.

Fat also serves as a flavor enhancer and gives a crunchy texture to the biscuit. The analysis of fat content in biscuits (control) was 8.12% and 9.00% in Bangun-bangun leaf biscuits. Most of the fat in biscuits comes from margarine and eggs because these two components have a fairly large fat content. According to Muchtadi And Sugiono [15], the chemical composition of eggs in 100 g contains fat in egg yolks as much as 34.5 g and margarine in 100 g fat content as much as 81 g. This shows that eggs and margarine play a role in the high-fat content produced in the manufacture of biscuits. Determination of fat content was carried out using the Soxhlet method.

Analysis of protein content in biscuits (control) was 8.98% and in Bangun-bangun leaf biscuits was 10.68%. Research conducted by Regia [16] the protein content in the SI group (control pie) was 10.27% and in the S2 group (pie with leaf substitution) 13.81%. The results of research conducted by Dewi [17] that the protein content of the F1 formula was 10.52% (bk) higher than the control formula 9.06% (bk). The protein content of the Bangun-bangun leaf biscuits was higher than the control biscuits because the leaves had high protein. Based on the Formula Quality Requirements according to the National Standardization Agency (2011) which states that the protein content is at least 5%, both the control formula and the Bangun-bangun leaf biscuits can meet these requirements.

Carbohydrates have an important role in determining the characteristics of foodstuffs. For example taste, color, texture, and others [18]. Analysis of carbohydrate content in biscuits (control) was 75.44% and in Bangun-bangun leaf biscuits was 74.94%. This indicates that the carbohydrate content of the control biscuits was higher than the Bangun-bangun leaf biscuits. Carbohydrate content in biscuits is influenced by water content, ash content, protein content, and fat content.

c. Antioxidant Activity of Bangun-bangun Leaf Biscuits

The results of the analysis of antioxidant activity on Bangun-bangun leaf biscuits can be seen in Table 4.

Table 4. Antioxidant activity on Bangun-bangun leaf biscuits

Sample	IC_{50} (µg/ml) ± SD
Bangun-bangun leaf biscuits	199,96 ± 11,52

The antioxidant activity (IC_{50}) of Bangun-bangun leaf biscuits was 199.96 µg/ml. The antioxidant activity of Bangun-bangun leaf biscuits decreased when compared to the antioxidant activity of Bangun-bangun leaf extract, ranging from 63.53 µg/ml – 77.55 µg/ml. Based on the standard level of antioxidant activity, compounds that are categorized as very active/high have IC50 values < 10 µg/ml, active/moderate categories if they have IC_{50} values of 10-100 µg/ml, and IC50 values > 100 µg/ml are categorized as inactive/ low [19]. The decrease in antioxidant activity occurs due to the process of making a mixture of ingredients and leaves that are not evenly distributed or the cooking process is not the same so that it can affect antioxidant activity [20]. In addition, the decrease in the activity of the leaf biscuits occurred due to the processing.

IV. Conclusion

Inactive/weak antioxidant activity was produced in A2B3 treatment with an average value of 77.55 µg/ml, while active/moderate antioxidant activity was produced by A1B1 treatment with an average value of 63.53 µg/ml. Bangun-bangun leaves extract with positive temperature and time variants contained flavonoid compounds and negative for steroidal alkaloids, for saponins there were positive and negative results in the extract of Bangun-bangun leaves with drying temperatures of 60 ^{o}C for 150 minutes and 80^{o}C for 120 minutes. The results of the proximate analysis of biscuits showed that the moisture content of the control biscuits was 4.69%, the Bangun-bangun leaf biscuits were 4.38% and the protein content of the control biscuits were 8.98%, the Bangun-bangun leaf biscuits were 10.68 and met the Quality Requirements for the Biscuit Formula according to SNI 2973. :2011.

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