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Formulation and Antioxidant Activity of Effervescent Granule Preparation of Bay Leaf Extract (Syzygium Polyanthum (Wight) Walp.) Using the DPPH Method

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Abstract
Excessive free radicals in the body may trigger oxidative stress associated with various degenerative diseases. Bay leaf (Syzygium polyanthum (Wight) Walp.) contains bioactive compounds, including flavonoids, saponins, and tannins, that possess antioxidant potential and were formulated into effervescent granules to improve practicality and consumer acceptance. This study aimed to formulate the ethanolic extract of bay leaf into effervescent granules meeting pharmaceutical physical quality standards and to evaluate their antioxidant activity using the DPPH method. This experimental laboratory study included plant determination, simplicia preparation, maceration extraction with 70% ethanol, phytochemical screening, and preparation of effervescent granules in four formulas (F0, F1, F2, F3) containing 0%, 0.5%, 0.75%, and 1% extract, respectively. Evaluation covered physical quality tests (organoleptic, flow rate and angle of repose, dissolution time, pH, moisture content, hedonic test, and physical stability) and DPPH antioxidant activity testing. All formulas met the physical quality requirements for good granules. The IC50 value of the ethanolic bay leaf extract was 82.213 ppm (strong category), while the IC50 values of the effervescent granules for F0, F1, F2, and F3 were 105.097; 71.490; 75.938; and 77.939 ppm, respectively. The ethanolic extract of bay leaf can be successfully formulated into effervescent granules that meet good physical quality standards and exhibit strong antioxidant activity.

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1. INTRODUCTION

Free radicals are reactive compounds with unpaired electrons in their outer shell. Excessive presence in the body can trigger various degenerative conditions. The body actually has an endogenous antioxidant defense system to regulate free radicals, but excessive free radical formation reduces this defense capacity, leading to oxidative stress, an imbalance between free radical formation and the antioxidant defense system that can damage cells and organs.

Consuming adequate amounts of supplemental antioxidants can reduce the risk of cardiovascular disease, cancer, atherosclerosis, osteoporosis, and other degenerative disorders. On the other hand, the use of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) in food products is beginning to be restricted due to their carcinogenic potential and the risk of liver damage. This situation has prompted the search for natural antioxidant sources as safer alternatives.

Bay leaves (Syzygium polyanthum) are a plant widely used as a cooking spice and also have therapeutic potential, including helping to treat digestive disorders, hypertension, diabetes mellitus, and diarrhea. This potential is related to the content of bioactive compounds such as flavonoids, tannins, and phenolic compounds, which have high antioxidant activity. Several

previous studies reported IC50 values for bay leaf extracts of 32.594 ppm, 15.45 ppm, and 18.36 ppm, respectively, all of which are in the very strong category.

To improve practicality and consumer acceptance, bay leaf extract needs to be formulated into a modern dosage form. Effervescent granules are coarse powder preparations that produce carbon dioxide gas when dissolved in water through an acid-base reaction, thereby helping the dissolution process of the active substance, masking unpleasant tastes, providing a fresh sensation, and having relatively good storage stability compared to other oral preparations [4]. The antioxidant activity of the preparation can be evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which is a stable, dark purple free radical and is widely used because the procedure is simple, easy, and relatively fast. The principle of this test is based on the ability of antioxidant compounds to donate hydrogen atoms to DPPH, which is indicated by a color change from purple to yellow, with measurements at a wavelength of 515–517 nm using a UV-Vis spectrophotometer; the lower the IC50 value, the stronger the antioxidant activity.

Previous research on effervescent granules of bay leaf extract has only focused on the antidiabetic aspects and physical quality evaluation [9], so the antioxidant potential of the preparation has not been proven specifically using the DPPH method. Based on this, this study was conducted to formulate ethanol extract of bay leaves into effervescent granules and evaluate its physical quality and antioxidant activity, in order to prove that the antioxidant activity of the natural ingredient remains stable after being formulated into a modern dosage form.

2. RESEARCH METHODS

This research is an experimental laboratory study aimed at developing an effervescent granule formulation based on bay leaf extract (Syzygium polyanthum) and testing its antioxidant activity using the DPPH method. The research was conducted for three months at the Natural Materials Laboratory of the Undergraduate Study Program of Pharmacy, Faculty of Health Sciences, Duta Bangsa University, Surakarta. The independent variable of the research was the concentration of bay leaf extract in formulas F0 (without active substance), F1 (0.5%), F2 (0.75%), and F3 (1%), while the dependent variable was the antioxidant activity of the effervescent granule preparation.

The raw material in the form of fresh bay leaves was obtained from the Polokarto area, Sukoharjo, Central Java, and was identified at the UPF Yankestrad RSUP Dr. Sardjito to ensure the accuracy of the species identity. The main tools used include a blender, oven, water bath, analytical balance, 16 and 20 mesh sieves, pH meter, moisture analyzer, flow tester, UV-Vis spectrophotometer, and rotary evaporator. The materials used include bay leaf simplicia, sodium bicarbonate, citric acid, tartaric acid, PVP K-30, sodium benzoate, mannitol, 70% ethanol and ethanol p.a., DPPH powder, and vitamin C p.a. as a comparison.

Preparation of Simplicia and Extract

The sorted and washed bay leaves were dried in the sun with a dark cloth cover, then ground and sieved with a 20-mesh sieve to obtain the simplicia powder [9]. The simplicia was standardized through drying shrinkage tests, water content, and ash content. Extraction was carried out using the maceration method with 70% ethanol solvent (powder-to-solvent ratio 1:10) for three days with periodic stirring; then the macerate was filtered, and the filtrate was concentrated using a rotary evaporator followed by a water bath at 50°C until a thick extract was obtained. The extract was then standardized, including water content, alcohol-free test, and metal contamination test (Pb and Cd), as well as screening its phytochemical content (alkaloids, flavonoids, saponins, tannins, and steroids) using color tests and typical precipitation of the compound group.

Effervescent Granule Formulation

The effervescent granule formula was developed based on the formula reference of Dwi Akbar et al. with modifications to the extract concentration, as presented in Table 1.

Table 1. Effervescent Granule Formula of Bay Leaf Extract

Material	Function	F0	F1	F2	F3
Bay leaf extract	Active ingredient	-	0,5 %	0,75 %	1%
Citric acid	Source of acid	2%	2%	2%	2%
Tartaric acid	Source of acid	19 %	19 %	19%	19 %
Sodium bicarbonate	Language source	31 %	31 %	31%	31 %
PVP K-30	Fastener	2%	2%	2%	2%
Natrium benzoat	Preservative	0,1 %	0,1 %	0,1 %	0,1 %
Dye	Dye	-	qs	qs	qs
MANNITOL	Sweetener/filler	ad 100	ad 100	ad 100	ad 100

Effervescent granules were prepared by separating the acid phase and the base phase. The base components (sodium bicarbonate, PVP, sodium benzoate, and dye) were stirred until homogeneous, sieved with a 16-mesh sieve, and then dried at 60°C for 15 minutes. The acid components (bay leaf extract, citric acid, and tartaric acid) were stirred until they formed a pliable mass, then sieved and dried in the same manner. The two granules were mixed and sieved again using a 16-mesh sieve before undergoing physical quality testing.

Evaluation of Physical Quality of Preparations

Evaluation of the physical quality of granules includes organoleptic tests (color, odor, and taste); flow rate and angle of repose test using the funnel method, with good flow rate criteria if the flow time of 100 g is ≤ 10 seconds and the angle of repose is ≤ 40°; dissolution time test with criteria ≤ 5 minutes; pH test using a pH meter with criteria 5–7; water content test using a moisture analyzer at a temperature of 105°C with criteria <4%; hedonic test on 20 panelists with a rating scale of 1 (very dislike) to 4 (very like) for color, odor, taste, and texture parameters; and physical stability test with the cycling test method for four cycles at a temperature of 4°C ± 2°C and 40°C ± 2°C, each 24 hours per cycle.

Antioxidant Activity Testing Using the DPPH Method

A 500-ppm DPPH solution was prepared by dissolving 50 mg of DPPH powder in absolute ethanol up to 100 mL, then determining the maximum wavelength (400–600 nm) and its operating time. Vitamin C was used as a comparison at a series of concentrations of 2, 4, 6, and 8 ppm, while the extract was tested at concentrations of 1, 5, 10, and 15 ppm, and effervescent granule preparations were tested at concentrations of 10, 20, 30, and 40 ppm. A total of 4 mL of DPPH solution was reacted with 50 µL of test solution at each concentration, incubated for 30 minutes in a dark place, and then the absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer.

Data Processing and Analysis

The inhibition percentage is calculated using the formula % inhibition *=[(Control absorbance − Sample absorbance) / Control absorbance] × 100%*[18]. IC value$_{50}$determined

from the linear regression equation y = a + bx, with x as the sample concentration and y as the inhibition percentage, by substituting the value y = 50 to obtain IC_{50}= (50 − a)/b [18]. The smaller the IC value$_{50}$, the higher the antioxidant activity of a sample.

3. RESEARCH RESULTS AND DISCUSSION

A. Research Results

Plant identification at the UPF Yankestrad RSUP Dr. Sardjito confirmed that the sample used was indeed a bay leaf plant (Syzygium polyanthum (Wight) Walp.). The yield of the simplicia obtained was 33.5% of the wet weight, which fulfilled the requirements for a good simplicia yield (>10%) [10].

Table 2. Results of Standardization of Simplicia and Bay Leaf Extract

Parameter	Results (Mean ± SD)	Conditions	Reference
Drying shrinkage of simple drugs	4,653% ± 0,12%	< 10%	Ministry of Health of the Republic of Indonesia [11]
Water content of simple drugs	5% ± 0,10%	< 10%	Chandra & Lisnawati [12]
Ash content of simple drugs	10,394% ± 1,02%	< 16%	Musdalipah et al. [13]
Extract yield	11,4%	> 10%	-
Extract water content	7,78%	< 10%	Ministry of Health of the Republic of Indonesia [11]
Alcohol-free	No ester odor	No ester odor	-
Metal contamination (Pb, Cd)	Negative (no color change)	Free from contamination	-

The results of phytochemical screening showed that the ethanol extract of bay leaves contained flavonoids, saponins, and tannins, but no alkaloids or steroids were detected in the color and precipitation tests carried out.

Table 3. Results of Physical Quality Tests of Effervescent Granule Preparations

Parameter	F0	F1	F2	F3	Conditions
Flow rate (g/sec)	7,2 ± 2,56	6,4 ± 0,94	5,4 ± 0,39	4,8 ± 0,57	< 10 seconds/100 g
Angle of repose (°)	12,9 ± 6,9	25,9 ± 2,23	25,7 ± 0,37	23,5 ± 1,26	≤ 40°

Parameter	F0	F1	F2	F3	Conditions
Dissolving time (minutes)	0,95 ± 0,43	0,78 ± 0,23	1,06 ± 0,03	0,94 ± 0,23	≤ 5 minutes
pH	5,71 ± 0,33	5,86 ± 0,19	6,06 ± 0,02	5,95 ± 0,26	5–7
Water content (%)	0,70 ± 0,17	1,79 ± 0,12	1,19 ± 0,35	1,17 ± 0,26	< 4%
Average hedonic (%)	70,93 ± 4,62	77,50 ± 5,79	79,68 ± 3,35	79,06 ± 5,47	-

Physical stability tests during four cycling test cycles did not show significant changes in the shape, color, odor, or taste of the granules of the four formulas, which indicates good physical stability of the preparation.

Table 4. IC50 Values of Extracts, Comparators, and Effervescent Granule Preparations

Sample	IC50 (ppm)	Category
Vitamin C (comparator)	18,015	Very strong
Ethanol extract of bay leaves	82,213	Strong
Chrysanthemum Granules (comparison)	21,271	Very strong
Granule F0 (without extract)	105,097	Currently
Granule F1 (0.5%)	71,490	Strong
Granule F2 (0.75%)	75,938	Strong
Granul F3 (1%)	77,939	Strong

B. Discussion

The yield of the simplicia of 33.5% and the yield of the extract of 11.4% have met the minimum requirements (>10%), which indicates the effectiveness of the pollination and extraction process in extracting active compounds; the higher the yield obtained, the greater the suspected content of active compounds in the sample. The drying loss value (4.653%), the water content of the simplicia (5%), and the ash content (10.394%) are all below the maximum required limits, indicating that the simplicia used have met quality standards and are not at high risk of microbial growth or external contamination during storage. Similarly, the water content of the extract of 7.78% and the negative results of the alcohol-free and metal-contamination tests confirm that the resulting extract is sufficiently pure and safe for use in the next formulation stage.

Phytochemical screening results showed that bay leaf extract contains flavonoids, saponins, and tannins, compounds that generally act as free radical scavengers through a hydrogen atom donor mechanism [3]. The absence of alkaloids and steroids in this test is thought to be related to the incompatibility of the polarity of the solvent used for these groups of compounds, rather than indicating the absolute absence of these compounds in the plant.

Physical quality evaluation showed that all four effervescent granule formulas met all required criteria. Flow rates and angles of repose within the required range indicated good granule flow properties, which are essential for dosage uniformity during packaging.

Dissolution times for all formulas were well below the maximum limit of 5 minutes, reflecting a rapid and complete effervescent reaction. The pH value in the range of 5.71–6.06 is considered safe against gastrointestinal irritation, while the low water content (<4%) reduces the risk of granule clumping and microbial growth during storage. In the hedonic test, Formula 2 (0.75% extract concentration) achieved the highest level of acceptance because it provided a balance of color, aroma, and a moderate sour taste, while the results of the physical stability test showed no organoleptic changes, confirming the stability of the formulas during accelerated storage.

In the antioxidant activity test, vitamin C as a comparison showed an IC50 value of 18.015 ppm (very strong category), while the ethanol extract of bay leaves showed an IC50 value of 82.213 ppm, which is classified as a strong category (range 50–100 ppm). This value confirms that the phenolic and flavonoid compounds in the extract play an important role in inhibiting DPPH free radicals through hydrogen atom donation, although its activity is not as strong as vitamin C as a pure synthetic antioxidant.

In the effervescent granule preparation, formula F0 without the addition of extract showed an IC50 value of 105.097 ppm (moderate category), while F1, F2, and F3 were 71.490; 75.938; and 77.939 ppm, respectively (all in the strong category). The significant decrease in the IC50 value from F0 to F1 proves that the addition of bay leaf extract makes a major contribution to the antioxidant activity of the preparation. However, the increase in the extract concentration in F2 and F3 was not followed by a consistent decrease in IC50, but rather a slight increase compared to F1. This trend is thought to be related to the interaction between formula components at higher extract concentrations, which can reduce the efficiency of the active compound in neutralizing free radicals. When compared with the comparison herbal granules based on chrysanthemum flowers, which have an IC50 of 21,271 ppm (very strong category), the four effervescent granule formulas of bay leaves in this study are still in the lower category, but still show that the antioxidant activity of bay leaf extract is relatively stable after going through the formulation process into effervescent granule preparations.

4. CONCLUSION

Based on the results of the research that has been conducted, it can be concluded that 70% ethanol extract of bay leaves (Syzygium polyanthum (Wight) Walp.) has antioxidant activity in the strong category with an IC50 value of 82.213 ppm. The effervescent granule preparation of bay leaf extract also has antioxidant activity in the strong category, with IC50 values of Formula 0, I, II, and III of 105.097; 71.490; 75.938; and 77.939 ppm, respectively. Bay leaf extract has been proven to be able to be formulated into an effervescent granule preparation that meets good physical quality standards, including organoleptic tests, flow rate and angle of repose, dissolution time, pH, water content, hedonic, and physical stability. Further research is recommended to examine the effect of different extraction methods and compare the antioxidant activity of bay leaf simplicia from other regions of origin to enrich scientific data regarding the potential of this plant.

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