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Phytochemical Screening And Antioxidant Activity Of The Tea Combination Of Bay Leaves (*Eugenia Polyantha*) And Basil Leaves (*Ocimum Basilicum*)

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ABSTRACT

Background: Hypertension is a non-communicable disease that is a major cause of morbidity and mortality in Indonesia. Hypertension is a key risk factor for various non-communicable diseases, such as cardiovascular, coronary heart disease, stroke, and chronic renal failure. Until now, the use of antihypertensive medications is the main method of treating hypertension. Indonesian natural plants can be used as an alternative treatment for hypertension. One of the natural plants, namely bay and basil leaves, can be developed to improve antihypertensive treatment because of their antioxidant activity. This study aimed to determine the phytochemical profile, antioxidant activity, and organoleptic tests of the combination of bay and basil leaves tea in three formulations. **Methods**:This type of research is quasi-experimental. The phytochemical profile at a parameter of alkaloids, flavonoids, tannins, saponins, and steroids was determined by qualitative tests. Antioxidant activity was determined by the DPPH method, and organoleptic tests at a parameter of taste, color, and scent were determined by 30 panelists. Phytochemical screening tests showed that the sample contains alkaloids, flavonoids, tannins, and saponins. **Results**: The inhibition ability of the samples against DPPH free radical was expressed with an IC_{50} value of formulations I, II, and III, which were 68.240, 64.125, and 71.030 ppm, respectively. Based on the IC_{50} value, it was proved that the three formulations have antioxidant activity index (AAI) in the strong category. Formulation I of the combination of bay and basil leaves tea was the most popular formula based on the organoleptic test from 30 panelists.

Keywords: Combination Tea Of Bay And Basil Leaves, Phytochemical Screening, Antioxidant Activity, Organoleptic Test

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INTRODUCTION

Antioxidants are a group of compounds that can protect cells from damage caused by free radicals in cells. Antioxidants interact and stabilize free radicals through several mechanisms such as transferring electrons, donating hydrogen, reducing peroxides, quenching singlet oxygen and superoxide, and chelating metal ions and converting them into harmless molecules (Nemzer et al., 2019; Sridevi et al., 2018; Zehiroglu & Ozturk Sarikaya, 2019).

Many natural biological processes in our body generate free radical compounds that are harmful to cells, namely ROS and RNS (Kurutas, 2016; Nimse & Pal, 2015; Sharifi-Rad et al., 2020). On the other hand, increased exposure to the environment and higher levels of dietary xenobiotics also contribute to the increased production of ROS and RNS in the body (Nimse & Pal, 2015). Oxidative stress can occur when the amount of ROS in the body exceeds the amounts of antioxidants, where the excess will attack lipid components, proteins, and DNA (Zulaikhah, 2017). The interaction of various ROS sources can impact redox signaling and increase oxidative stress. Oxidative stress plays an important role in the development of many chronic diseases including cardiovascular disease, aging, heart disorders, anemia, cancer, and inflammation (Sharifi-Rad et al., 2020; Zehiroglu & Ozturk Sarikaya, 2019).

Oxidative stress can be effectively neutralized by enhancing cellular defenses in the form of antioxidants (Nimse & Pal, 2015). Antioxidants can inhibit free radicals through the mechanism of redox reactions. Oxidation reactions in the body can produce free radicals, which initiate a chain reaction that damages cells. Antioxidants are able to end this chain reaction by removing free radical intermediates and inhibiting other oxidation reactions by oxidizing themselves (Hamid et al., 2010). Under excessive oxidative stress, the natural human antioxidant system cannot neutralize the damaging effects of free radicals that can damage cellular DNA, lipids, proteins, and other biomolecules leading to the development of chronic diseases and premature aging. Therefore, exogenous antioxidants are required to scavenge free radicals in the body and prevent their actions in vivo to protect cells and tissues. (Nemzer et al., 2019).

Antioxidants can be classified into two basic groups, namely synthetic and natural. Natural antioxidants are generally derived from plant sources and their activity varies depending on the plant species, diversity, extraction and/or processing methods, and growing conditions (Zehiroglu & Ozturk Sarikaya, 2019). Compared to synthetic antioxidants, natural antioxidants from plants are considered more acceptable, reliable, and safer, to promote health and prevent disease (Zhang & Gao, 2014).

Some previous studies have proven that various types of natural ingredients such as gamal leaves, garlic, lemon, and cemcem leaves contain bioactive compounds with various pharmacological activities, one of which is antioxidant (Artaningsih et al., 2018; Bekti et al., 2022; Dewi et al., 2020; Dharmawati et al., 2022; Vinenthy et al., 2019). Other natural ingredients that were reported to contain bioactive compounds with antioxidant potential are bay leaves (*Eugenia polyantha*) and basil leaves (*Ocimum basilicum*). Some studies state that bay leaves and basil leaves contain various secondary metabolite compounds, such as alkaloids,

flavonoids, tannins, and saponins with various pharmacological activities including antibacterial and antioxidant (Norihsan & Megantara, 2018; Tatiana & Ria, 2020).

The use of bay leaves and basil leaves has been limited as ingredients in various traditional dishes. Whereas, the content of various secondary metabolite compounds in both leaves has the potential to develop as a traditional medicine. This exploration can be carried out with various forms, such as simplicia, extracts, and other forms that are suitable for their intended use. A combination tea of bay leaves and basil leaves as a simple preparation can be made to optimize the benefits of both leaves. This form will make it easier for people to optimize the bay leaves and basil leaves as herbal products that are beneficial for health. The combination of bay leaves and basil leaves as a tea product would improve the taste and increase their pharmacological activity. However, the potential of bay leaf and basil leaf combination tea as a natural antioxidant has not been widely studied. Therefore, this research will study the phytochemical screening, antioxidant activity, and organoleptic tests on the combination of bay and basil leaves tea as an alternative for antioxidant natural products.

METHOD

This research is descriptive research. In this study, samples were tested without being randomly selected. The bay and basil leaves used in this study were obtained from Jegu Village, Tabanan, Bali. The samples used in this study were combination tea made by mixing the powdered simplicia of bay and basil leaves in formulations I (1:1), II (1:2), and 3 (2:1). Subsequently, the combination tea samples were brewed using 100 mL of water at 70˚C for 5 minutes. Furthermore, phytochemical screening testing was carried out at a parameter of alkaloid, flavonoid, tannin, saponin, and steroid, antioxidant activity test with DPPH method by UV-Vis spectrophotometry and organoleptic test at a parameter of taste, color, and scent using 30 panelists. The tests were conducted at the Laboratory of Warmadewa University, in January-March 2023.

Furthermore, the data obtained were recorded, processed, presented in a tabulation, and narrated and discussed in accordance with the theory and related literature. This research has been reviewed and obtained Ethical Approval from the Health Research Ethics Commission of the Denpasar Health Polytechnic Number: LB.02.03/EA/KEPK/0032/2023.

Preparation of The Tea Combination of Bay and Basil Leaves

The clean and fulfilled criteria of bay leaves and basil leaves were dried by using an oven at 50˚C for 20-30 hours. Next, the dried leaves were ground with a blender to obtain the fine powder. Furthermore, each powder was weighed and mixed to obtain 3 formulations, namely formulation 1 (1:1), formulation 2 (1:2), and formulation III (1:3) with the total mass of each formulation being 3 grams. For the examination process, each combination tea sample was brewed using 100 mL of water at 70˚ for 5 minutes.

Phytochemical Screening of The Tea Combination of Bay and Basil Leaves

Alkaloids: 1 mL of brewing tea added with N-ammonia-chloroform solution. Furthermore, the mixture was shaken for 1 minute and then filtered. Next, 5 mL of H_2SO_4 was added and shaken. After settling, separate

the aqueous phase and test it by adding Mayer's reagent (Habibah & Ratih, 2023).

Flavonoids: 5 mL of the brewing tea was put into a glass beaker, then added with 10 mL of ethyl acetate, then boiled and filtered. Furthermore, 0.5 mL of the filtrate was added with 1 mL of dilute ammonia solution, then observed the changes that occurred in the sample (Habibah & Ratih, 2023).

Tannins: A total of 1.6 mL of brewing tea was added to the $FeCl_3$ solution. Subsequently, the color changes were observed (Habibah & Ratih, 2023).

Saponins: A total of 10 mL of brewing tea was added to 5 mL of distilled water and then shaken vigorously until foam formed. Then 3 drops of olive oil were added, after which it was shaken again and observed for the formation of an emulsion (Habibah & Ratih, 2023)

Steroids: A total of 2 mL of brewing tea was evaporated in a porcelain cup. The residue is dissolved with 0.5 mL of chloroform, then 0.5 mL of anhydrous acetic acid is added. Then 2 mL of concentrated sulfuric acid was added through the tube wall. The formation of a brownish or violet ring at the boundary of the solution indicates the presence of triterpenoids, whereas a greenish-blue ring appears indicating the presence of steroids (Habibah & Ratih, 2023).

Antioxidant Activity of The Tea Combination of Bay and Basil Leaves

A series concentration of the brewing tea (30, 60, 90, 120 and 140ppm) was prepared. 1 mL sample was added to 1 mL of 40ppm DPPH solution in methanol. The mixed solution was then vortexed and incubated for 30 minutes at 27˚C. The absorbance of the solution was then measured at 516 nm. Antioxidant activity was expressed by the percentage of inhibition (IC_{50}), which was calculated based on = [(AC-AS)/AC] x 100%, where AC = control absorbance, and AS = sample absorbance (Andarwulan et al., 2010; Widyawati et al., 2012).

Organoleptic Test of The Tea Combination of Bay and Basil Leaves

The organoleptic at a parameter of taste, color, and scent was conducted by using 30 panelists. The organoleptic test was carried out by tasting a small amount of the brewing tea on the three formulations as a sample. After tasting each formula, the panelist neutralized their sense of taste by drinking a little mineral water. Subsequently, panelists were asked to fill in the organoleptic test results instrument using a 5-level Likert scale (Ratih & Habibah, 2022).

RESULTS

Phytochemical Screening of The Tea Combination of Bay and Basil Leaves

The results of the phytochemical screening test of the combination tea of bay and basil leaves showed that the samples contained various phytochemical compounds such as alkaloids, flavonoids, tannins, and saponins. The qualitative test results are presented in Table 1.

Table 1. Qualitative Test Result of Phytochemical Compounds on the Tea Combination of Bay and Basil Leaves

Phytochemical compounds	Observation Result	Interpretation
Alkaloid	Red precipitate was formed	+
Flavonoid	Orange color was appeared	+
Tannin	Greenish-brown color was appeared	+
Saponin	Foam was formed	+
Steroid	No color change	-

The antioxidant activity of the brewing tea of the combined bay and basil leaves samples was carried out through several test stages, namely the measurement of the maximum wavelength, the determination of the inhibition curve of each formulation, and the determination of antioxidant activity which is expressed by an IC_{50} value. The measurement of the maximum wavelength was carried out using a DPPH solution with a concentration of 40 ppm in the wavelength range of 400-700 nm. In this study, the maximum wavelength was obtained at 516 nm with an absorbance of 0.982. The maximum wavelength measurement is presented in Figure 1.

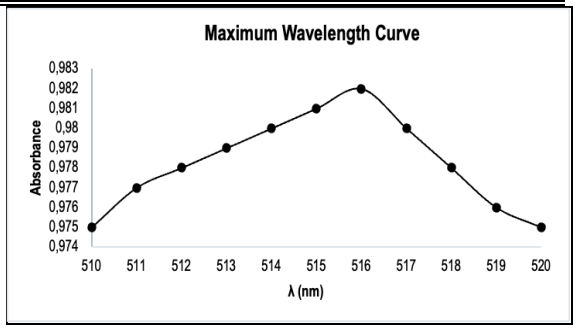

Figure 1. Maximum Wavelength Curve

Antioxidant Activity of The Tea Combination of Bay and Basil Leaves

The antioxidant activity determination of the three formulations initially starts with the measurement of the absorbance of the samples. Absorbance measurement was carried out with three repetitions in the concentration series of sample solution (30, 60, 90, 120, and 140 ppm). Furthermore, the % inhibition of the sample was calculated based on the absorbance of the sample and blank solutions. Based on the calculation results and the average % inhibition of each formulation, the linear regression equation was obtained as shown in Figure 2-4.

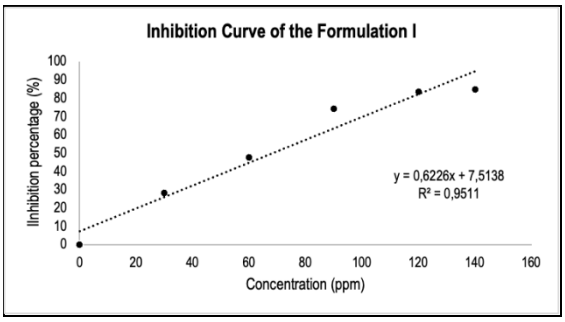

Figure 2. Inhibition Curve of the Formulation I

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Based on Figure 2, a linear regression equation was obtained of y = 0.6226x + 7.5138 with a correlation coefficient (r) of 0.9511. So, the IC_{50} value was obtained at 68.240 ppm.

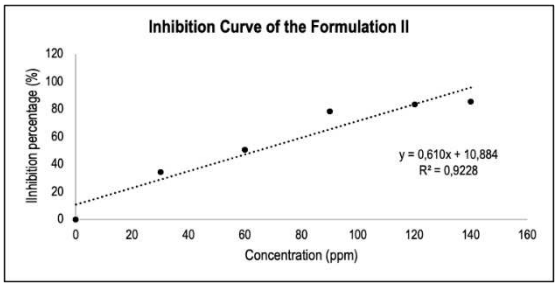

Figure 3. Inhibition Curve of the Formulation II

Based on Figure 3, a linear regression equation was obtained of y = 0.610x + 10.884 with a correlation coefficient (r) of 0.9228. So, the IC_{50} value was obtained at 64.125 ppm.

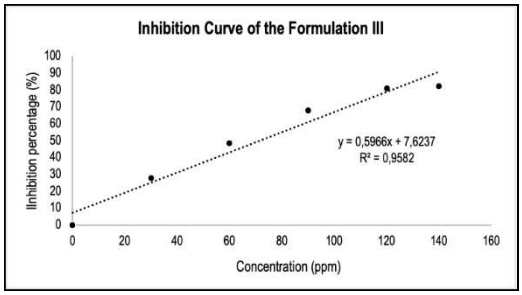

Figure 4. Inhibition Curve of the Formulation III

Based on Figure 4, a linear regression equation was obtained of y = 0.5966x + 7.6237 with a correlation coefficient (r) of 0.9582. So, the IC_{50} value was obtained at 71.030 ppm.

Furthermore, the antioxidant activity index (AAI) of the samples was calculated based on the IC_{50} value obtained for each formulation. The antioxidant activity of the samples was classified into the category of very weak to very strong. Based on the comparison of the DPPH concentration with the IC_{50} value of the samples, it is shown that the three formulations have antioxidant activity in the strong category. The classification of the antioxidant activity category of the samples is presented in Table 2.

Table 2. Category of the Antioxidant Activity on the Tea Combination of Bay and Basil Leaves Based on an IC_{50} Value

Formulation	IC_{50} (ppm)	AAI	Interpretation
I	68.240	0,586	Strong
II	64.125	0,624	Strong
III	71.030	0,563	Strong

Organoleptic Test of The Tea Combination of Bay and Basil Leaves

The organoleptic test result by using 30 panelists is represented using a 5-level Likert scale, which is very poor to excellent. The result of the organoleptic test with a Likert scale is shown in Table 3.

Table 3. Results of The Organoleptic Test with a Likert Scale.

Parameter	Likert Percentage (%)		
	Formulation I	Formulation II	Formulation III
Taste	**100**	40	70
Color	**20**	20	20
Scent	**76.7**	40	66.7

The following are the criteria for interpreting the scores based on intervals:
0% – 19.99% = Very poor; 20% – 39.99% = Poor; 40% – 59.99% = Fair / Neutral; 60% – 79.99% = Good/Like; 80% – 100% = Excellent

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DISCUSSION

Phytochemical Screening of The Tea Combination of Bay and Basil Leaves

Alkaloids

Alkaloids are a class of secondary metabolite compounds that are widely distributed and found in various types of plants. Alkaloids have promising pharmacological activities so they are often used in medicine (Habibah & Ratih, 2023). In this study, qualitative tests of alkaloid group compounds were carried out using Dragendorf Reagent. Based on the observation of the test results, it was formed a red precipitate in all of the samples. The precipitate formed is potassium alkaloid. The formation of a precipitate in the samples is due to the result of the reaction between the alkaloid compounds in the sample with the tetraiodobismutat(III) ions in the Dragendorf Reagent (Sulistyarini et al., 2019).

Flavonoids

Flavonoids are the largest class of phenol compounds that are found in plants. Flavonoids are a class of secondary metabolite compounds that can dissolve in water and are easily extracted with ethanol. Based on the qualitative test results, it is known that the three samples are positive for flavonoids as indicated by the color change of the sample solution to yellow-orange. The change in the color of this sample occurs because flavonoids in the form of phenol compounds can change color if a base or ammonia is added due to conjugation in their aromatic structure (Habibah & Ratih, 2023; Indarto, 2015; Kharismawati, M; Utami, P.I.; Wahyuningrum, 2009).

Tannin

The results of the tannin qualitative test in this study showed positive results indicated by the color change of the sample to greenish brown. Tannin is a secondary metabolite compound that is polar and soluble in water due to the presence of OH groups in its structure. The addition of 1% FeCl3 solution will change the color to dark blue or brown/green-black which proves the presence of tannin compounds in the sample (Habibah & Ratih, 2023; Sulistyarini et al., 2019).

Saponins

The obtained results from the qualitative test showed that the three samples were positive for saponins. This is indicated by the formation of a stable foam on shaking the sample after the addition of hot water and 1N HCl solution. The glycoside content in saponins will undergo hydrolysis into glucose and other compounds, thus causing the formation of stable foam in the liquid after being shaken with water (Wilapangga & Sari, 2018).

Antioxidant Activity of The Tea Combination of Bay and Basil Leaves

In this study, the determination of the antioxidant activity of the samples was carried out by using the DPPH method spectrophotometrically. The initial stage in this method was carried out by measuring the maximum wavelength. Maximum wavelength measurement is conducted to determine the λ that has the highest absorbance with the best accuracy. Absorbance measurements are performed at the maximum wavelength because of their sensitivity, minimum measurement error, and linear correlation between absorbance and concentration, and under these conditions, the Lambert-Beer Law is fulfilled (Gandjar & Rohman, 2007). Wavelength measurements in this study were carried out using DPPH (1,1-diphenyl-2-picrylhydrazyl) solution at a concentration of 40 ppm in the range of 400-700nm by UV-Vis

spectrophotometry. Based on the results which have shown in Figure 1, it is known that the maximum wavelength was obtained at 516 nm with an absorbance value of 0.982.

The antioxidant activity of bay and basil leaves combination tea was measured quantitatively using the DPPH method. This method is based on the ability of the sample to reduce or capture the DPPH free radicals. The inhibitory ability of the sample against DPPH radicals is expressed by the IC_{50} value. The IC_{50} value was determined based on the linear regression equation obtained from the % inhibition curve presented in Figure 2-4. The % inhibition curve was obtained by measuring the absorbance of the sample solution series at 5 concentration variations, namely 30, 60, 90, 120, and 140 ppm at the addition of a constant volume of DPPH solution. Based on the measurement results, it is known that the greater the concentration of the sample, the greater the inhibitory ability of the sample against DPPH radicals. This is indicated by the decreasing color intensity of the DPPH solution at increasing sample concentration. The reduced color intensity of the DPPH solution indicates that there is a reaction between the hydrogen atoms released by the sample with the radical molecules contained in DPPH so that it can produce a yellow and more stable 1,1-diphenyl-2- picrylhydrazyl compound (Purwanti et al., 2019). The inhibition ability of the samples against DPPH free radical was expressed with an IC_{50} value of formulations I, II, and III, which were 68.240, 64.125, and 71.030 ppm, respectively. Subsequently, the antioxidant activity index (AAI) of the samples was calculated based on the comparison of the DPPH concentration with the IC_{50} value of the samples. Furthermore, the antioxidant activity of the samples was then classified into the category of very weak to very strong. The result was shown that the three formulations have antioxidant activity in the strong category.

The antioxidant activity of the sample is closely related to the flavonoid compounds in the sample. Flavonoids which are a large group of phenolic compounds have characteristics in their aromatic structure because of the presence of hydroxyl groups in their ring structure. The antioxidant activity of the sample occurs because the hydroxyl group in the antioxidant compound will be donated to the free radical compounds, making them more stable and unreactive (Amponsah-Offeh et al., 2023; Habibah & Ratih, 2023).

Organoleptic Test of The Tea Combination of Bay and Basil Leaves

Organoleptic tests are widely used to assess quality of the food and beverage products. This test was conducted to determine the preference level of consumers at at parameters of taste, color, and scent. The preference test was conducted by asking the panelists after tasting the products. The panelist's response in the form of liking or disliking the properties of the material was recorded. In the assessment of food and beverages, the characteristic that determines whether a product is accepted or not is its sensory properties. The senses used in assessing the nature of the senses are the senses of sight, touch, smell, and taste, while the questionnaire is used as a tool in the form of a list of questions that must be filled out by the person (panelist) to be measured (Ratih & Habibah, 2022). The organoleptic test in this study was conducted to determine the selected formulation with the test method carried out, namely scoring based on the highest Likert percentage score. The results of

the organoleptic test obtained formulation I with the highest score on taste parameters (100%), color (20%), and scent (76.7%).

CONCLUSIONS

Phytochemical screening tests of the tea combination of bay and basil leaves showed that the sample contains alkaloids, flavonoids, tannins, and saponins. The inhibition ability of the samples against DPPH free radical was expressed with an IC_{50} value of formulations I, II, and III, which were 68.240, 64.125, and 71.030 ppm, respectively. Based on the IC_{50} value, it was proved that the three formulations have antioxidant activity index (AAI) in the strong category. Formulation I of the combination of bay and basil leaves tea was the most popular formula based on the organoleptic test from 30 panelists.

Conflict of Interest

During the research, there is no conflict of interest related to this research and publication.

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