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Research Article

ANALYSIS ANTIOXIDANT ACTIVITY AND TOTAL FLAVONOID CONTENT COMBINATION OF ETHANOL EXTRACT OF KENCUR RHIZOME (*Kaempferiae Galanga L.)* AND TAPAK DARA LEAF (*Catharanthus Roseus*) USING DPPH METHOD

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ABSTRACT

Background: Kaempferia galanga L and Catharanthus roseus leaves are natural ingredients that can be used as a source of antioxidants because they can reduce oxidative damage to the body due to free radicals. The aim of this research was to determine whether the ethanol extract of kencur rhizome and tapak dara leaves alone or combined has antioxidant properties that are able to fight free radicals.
Methods: The method used to test antioxidant activity uses the DPPH (2,2-diphenyl-1 picrylhydrazyl) method.
Results: the single ethanol extract of tapa dara leaves has antioxidant activity with an IC50 value of 32.17 ppm and is classified as very strong, while the single extract of kencur rhizome has antioxidant activity with an IC50 value of 17.30 ppm. and is classified as very strong. On the other hand, a mixture of ethanol extracts of tapak dara leaves and kencur rhizomes in a ratio of 1:2 showed very strong antioxidant activity, with an IC50 of 15.65 ppm. A mixture of tapak dara leaves and kencur rhizomes in a ratio of 2:1 showed very strong antioxidant activity with an IC50 value of 9.15 ppm. A mixture of galangal rhizome extract and tapak dara leaves in a ratio of 2:1 produces maximum antioxidant activity. This combination has very strong antioxidant activity compared to the individual forms of these compounds and is almost as strong as vitamin C which has an IC50 value of 5.30 ppm.
Conclusion: : the combination of galangal rhizome extract and tapak dara leaves in a ratio of 2:1 produces very strong antioxidant activity compared to the single form of both.

Keywords: Antioxidants; Kaempferia galanga L; Catharanthus roseus

INTRODUCTION

Molecules or atoms with one or more unpaired species are called free radicals. Free radicals are reactive, always trying to make electron pairs more stable. Cancer, coronary heart disease, stroke, several chronic and degenerative diseases that can be caused by free radicals in the body. Every time, the body experiences an oxidative reaction that produces radicals which can damage cell structure and function. However, the activity of free radicals can be reduced by the presence of antioxidants which can complement the immune system (1).

Antioxidants are chemical substances that are able to provide the electrons they contain to free radicals. The body can naturally produce antioxidant compounds, both in the form of enzymes and non-enzymes. However, these antioxidant compounds cannot completely inhibit the formation of oxidants that appear due to oxidative stress. Therefore, additional exogenous antioxidants are needed which can be obtained either synthetically or from natural sources (2).

Studies on natural antioxidants continue to be carried out, including in the field of medicinal plants. Medicinal plants contain herbal components that are rich in antioxidant compounds. Antioxidant compounds have the ability to fight or reduce the negative effects of oxidants in the body. They work by providing electrons to oxidized molecules, which prevents the oxidized compound from becoming active (3). Some natural compounds included in the antioxidant group are phenolic compounds such as gallic acid and flavonoids. Phenolic and flavonoid compounds have hydroxyl groups which can provide hydrogen atoms, thus acting as antioxidants and converting free radicals into more stable compounds (4).

Flavonoids are phenolic compounds with high antioxidant activity. Flavonoid compounds effectively remove oxidized compounds that can cause damage (5). Antioxidants are divided into two categories, namely synthetic antioxidants and natural antioxidants. The use of synthetic antioxidants is generally risky for human health due to their toxic properties if consumed in excess. Therefore, it is very important to use natural antioxidants that are good for health and do not have many side effects. Sources of natural antioxidants that come from plants are tapak dara leaves and galangal rhizomes. Utilization of the n-hexane and ethyl acetate fractions of the galangal rhizome plant resulted in IC50 antioxidant activity of 829.737 g/mL and 731.832 g/mL respectively, based on previous investigation findings (6)The tapak dara plant is an Indonesian plant with various contents such as alkaloids, flavonoids, phenolics, tannins and terpenoids with an IC50 antioxidant activity of 142,914 ppm (7). The high activity of these two plants makes these plants have potential as an alternative substitute for the use of synthetic antioxidants (8). In this study, ethanol extracts of kencur rhizomes and tapak dara leaves were combined to assess total flavonoid levels and antioxidant activity using the DPPH technique.

METHODS

Determination

Carried out at the Environmental Laboratory, Faculty of Biology, Jenderal Soedirman University, Purwokerto, to determine the identity of the plants to be used.

Qualitative Analysis of Flavonoids

Weighed 10 mg of the extract and added magnesium powder then added 5 drops of concentrated hydrochloric acid into the test tube. The formation of a yellow color in positive samples contains flavonoid compounds (9).

Flavonoid Quantitative Test

a. **Preparation of Quercetin Standard Solution Concentration Series**
   50 mg of quercetin was weighed and 96% ethanol was added to the mark in a 100 mL volumetric flask to obtain a standard solution of 500 ppm. Dilutions were made with concentration series of 2, 4, 6, 8, and 10 ppm.
b. **Determination of Maximum Wave Length**
   A 500 ppm solution was made, 1 mL pipetted, and reacted with 1 mL of 10% aluminum(III) chloride and 8 ppm quercetin. Measurements were then carried out using UV-Vis spectrophotometry in the wavelength range 300 - 500 nm (10).
c. **Measurement of Quercetin Standard Solution Absorbance**
   Pipette 1 mL of each standard solution that has been prepared, add 1 mL of 10% aluminum(III) chloride and 8 mL of 5% acetic acid (CH3COOH), and read the absorbance results at the maximum wavelength (10)
d. **DeterminationFlavonoid content of kencur rhizome extract, tapak dara leaf extract and combination of kencur rhizome: tapak dara leaves**
   1. Preparation of a stock solution of 1000 ppm each extract
      Weighed 25 mg of each extract, dissolved in 25 mL of ethanol (pa)
   2. Absorbance Measurement
      Pipette 1 mL of each 1000 ppm extract, add 3 mL of ethanol (pa); 1 mL AlCl3 10%; 8 mL of 5% acetic acid, and incubate the mixture at room temperature so that the reaction can occur. The absorbance of the solution was measured using the maximum wavelength and the measurement was repeated 3 times.

Antioxidant Activity Test

a. **Preparation of 40 ppm DPPH Solution**
   Added to a 100 mL volumetric flask, 4 mg of DPPH powder was dissolved in ethanol to the limit mark. Then, the mixture is carried out homogeneously.
b. **Determination of the maximum wavelength of DPPH**
   Add 2 mL of 40 ppm DPPH solution to the test tube with 2 mL of ethanol added and homogenize. The measurement wavelength range is 400-800 nm.
c. **Preparation of Blank Solution**
   Add 2 mL of 40 ppm DPPH solution and mix with 2 mL of ethanol into a test tube. Next, it was incubated for 30 minutes, the absorbance was read at the maximum wavelength.
d. **Preparation of Quercetin Standard Solution**
   Weighed 10 mg of quercetin and then mixed it with methanol using a 100 mL volumetric flask until a concentration of 100 ppm was obtained. Next, it is taken as 0.1; 0.2; 0.3; 0.4; and 0.5 mL of quercetin standard solution. After that, it was transferred to a measuring flask, and ethanol was added up to the limit mark to obtain several concentrations, namely 1, 2, 3, 4 and 5 ppm.
e. **Preparation of Extract Solution**

Weighed 10 mg of the extract and then put it into a volumetric flask by adding methanol up to the mark to obtain an extract solution at a concentration of 100 ppm. Then, the sample solution was taken as much as 0.5; 0.75; 1.125; and 1.5 mL of extract solution with a concentration of 100 ppm. The sample solution was then placed into a 10 mL volumetric flask, and methanol was added to the mark. Thus, concentration series 5; 7.5; 10, 12.5; and 15 ppm was obtained

f. Quercetin Standard Solution Antioxidant Test

From each set of prepared concentrations, pipette up to 2 mL of quercetin solution. Next, add 2 mL of 40 ppm DPPH sea. Then it was incubated and the absorbance was measured to obtain a standard curve for the quercetin standard solution

g. Antioxidant test of kencur rhizome extract, tapak dara leaves, and a combination of kencur rhizome and tapak dara leaves (1:1, 1:2, 2:1)

Extract solution with concentration series 5; 7.5; 10; 12.5; and 15 ppm as much as 2 ml is pipetted and placed in a test tube. Next, 2 ml of DPPH solution with a concentration of 40 ppm was added into the same test tube. The mixture is then homogenized properly and incubated so that it can be further absorbed at the maximum wavelength

RESULTS

Determination of the maximum wavelength between 300 – 500 nm with a UV-Vis spectrophotometer to measure the absorption of a standard quercetin solution. The aim of this procedure is to determine the area of absorption that can be produced (11). The maximum wavelength of quercetin obtained was 423 nm. A standard curve was initially created to determine the concentration of flavonoids in the samples based on the absorbance obtained. This procedure involves creating a standard curve by measuring the absorbance of a standard solution of quercetin that has been previously diluted in stages. Serial dilution solutions of standard quercetin solutions are prepared carefully and carefully to achieve the desired concentration. This precaution is intended to prevent errors that allow the concentration of the standard solution to be inconsistent with the results achieved (12).The standard curve was determined using quercetin standards at concentrations of 2, 4, 6, 8, and 10 ppm, then the absorbance was read at 423 nm. The results of determining the absorption of quercetin standard curve measurements can be seen in Table 1.

Table 1. Absorbance results of quercetin standard curve measurements

Concentration (ppm)	Absorbance at a wavelength of 423 nm
2	0.235
4	0.303
6	0.374
8	0.440
10	0.520

Total flavonoid levels were determined by entering the absorbance values

of kencur rhizome extract and tapak dara leaf extract into the quercetin standard curve equation. The results obtained from this determination are then displayed in Table 2.

Table 2. Total flavonoid content of extract

Extract	Total Flavonoid Content (mgQE/g extract)	Average Total Flavonoid Content (mgQE/g extract)
Kencur Rhizome	9.70 9.84 9.84	9.80
Tapak Dara leaves	5.77 6.62 5.57	5.99
Combination (2:1)	11.54 11.66 11.66	11.62

The antioxidant activity of each extract and combination of extracts was evaluated through a comparison between ethanol extract from kencur rhizome and ethanol extract from tapak dara leaves with a ratio of (1:1), (1:2), and (2:1). Data on the results of this antioxidant activity test are listed in detail in Table 3.

Table 3. Results of extract antioxidant activity tests

Sample	IC50 (ppm)	Information
Kencur Rhizome	17.30	Very strong
Periwinkle leaves	32.17	Very strong
Combination of galangal rhizome and tapak dara leaves (1:1)	20.32	Very strong
Combination of galangal rhizome and tapak dara leaves (1:2)	15.65	Very strong
Combination of galangal rhizome and tapak dara leaves (2:1)	9.15	Very strong

DISCUSSION

Plant determination is carried out to ensure the authenticity of the kencur rhizome and periwinkle leaves so that errors in plant use do not occur. Determination was carried out at the Faculty of Biology, Jenderal Soedirman University, Purwokerto. The results of the determination proved that the plants used were indeed kencur rhizomes and tapak dara leaves.

The qualitative flavonoid test aims to identify flavonoid compounds in each extract. This test is carried out by taking 10 mg of the extract and mixing it with magnesium (Mg) powder. Next, 5 drops of concentrated HCl were added to the mixture. This process aims to produce red or orange flavilium salts. The benzopyrone core contained in the flavonoid structure is reduced by adding Mg and HCl powder to the flavonoid test (13). The test results showed that tapak dara leaf extract and kencur rhizome extract both contained flavonoids based on the color change to red or orange.

Based on Table 1, the concentration value and absorbance value are directly related, with a high concentration value producing a higher absorbance value. The equation y = 0.0353x + 0.1623 with a correlation coefficient (r) of 0.9995 is a linear regression value based on the findings of absorbance measurements. The numerical measure of how strong, moderate, or weak the relationship is between the variables under study is the correlation coefficient (r). The correlation coefficient (r) value shows a high level of confidence and the curve becomes linear closer to 1 (12).

The colloimetric method with AlCl3 reagent was used to determine the total flavonoid content. The production of stable compounds containing C-4 keto groups as well as C-3 or C-5 hydroxyl groups from flavones and flavonols is the basis of the approach. In addition, the protohydroxyl group on the A or B ring of the flavonoid compound will combine with aluminum chloride to produce a stable acid complex (12). Because quercetin belongs to the flavonoid group of flavonols and has a keto group at the nearest C-4 and C-5 atoms, quercetin is the molecule used as a standard in determining flavonoid concentrations (14).

The combination of ethanol extract of kencur rhizome plants and tapak dara leaves in a ratio of 2:1 has the highest total flavonoid content, while ethanol extract of tapak dara leaves has the lowest flavonoid content. Apart from that, the combination of the two extracts in a ratio of 2:1 can also increase the total flavonoid content. Its antioxidant activity shows its ability to capture free radicals. In this study, the DPPH (2,2-diphenyl-1-picrylhidrazyl) technique was used because it is the most common method for measuring antioxidant activity (15). The IC50 value, or the concentration of a test substance that has the ability to reduce 50% of free radicals, is calculated through antioxidant activity testing. The IC50 value is calculated using the linear regression equation Y = bx + a, and the smaller the IC50 value, the more free radical scavenging activity (16).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is a popular in vitro approach for determining antioxidant activity. This method was chosen because it was considered easy, fast, sensitive, and only required a small sample. By using the DPPH compound, free radical scavenging reactions can be observed, which allows assessing the

antioxidant activity of the tested compounds. Only stable DPPH molecules and comparators such as vitamins A, C, and E are required for this approach. Additionally because free radicals can easily replace the substrate in this process, a substrate is not required (17). The working principle underlying the DPPH method is based on the capacity of DPPH to accept a given hydrogen atom. The color of DPPH can change from the initial color (purple) to yellow when an antioxidant is present or when one element in DPPH pairs with the antioxidant's hydrogen (16).

Determination of the maximum wavelength is carried out by measuring the absorption of a standard DPPH solution which has a concentration of 40 ppm in the wavelength range of 400 - 800 nm. The goal of this step is to maximize the absorption of light by the solution by identifying the point where the absorption reaches its highest value (18). Based on the measurement results, it was found that the DPPH solution showed maximum absorption at 516 nm. Therefore, antioxidant activity tests were carried out at this wavelength. The use of the maximum wavelength was chosen because at this value, the solution has the highest sensitivity to changes in absorbance for each unit concentration. Thus, the information obtained about the antioxidant activity of the test substance will be more accurate (19).

Based on the antioxidant test results, quercetin is included in the very strong category with an IC50 value of 4.23 ppm > 50 ppm. Quercetin was chosen as a comparison solution because it is the most widely distributed compound found in plants (20). The results of the quercetin antioxidant activity test obtained were in line with research conducted byPujiastuti & Ma'rifah, (2022)where the IC50 value of quercetin obtained was 7,640 ppm.

Based on the antioxidant activity test results data listed in Table 3, it can be seen that the ethanol extract of kencur rhizome shows superior antioxidant activity when compared to the ethanol extract of tapak dara leaves. Both the ethanol extract of kencur rhizome and its combination form with the ethanol extract of tapak dara leaves at ratios of (1:1), (1:2), and (2:1) showed antioxidant activity values that were included in the very strong category.Hayati et al., (2016)stated that with an IC50 value of 13.07 mg/m, the ethanol extract of galangal rhizome can function as a very potent DPPH radical inhibitor. With a combination ratio of (1:1) and (1:2), the combination of ethanol extract of kencur rhizome and tapak dara leaves (2:1) has the greatest antioxidant activity value, namely 9.15 ppm. This is because free radical scavenging activity increases with decreasing IC50 value (16).

It is estimated that secondary metabolite compounds such as phenolics and flavonoids, which are found in tapak dara leaves and kencur rhizomes, significantly reduce the activity of DPPH radicals. These compounds are thought to have the ability to effectively inhibit DPPH radicals. The phenolic and flavonoid groups have been proven to have strong antioxidant properties, so they have the potential to be the main factor in inhibiting DPPH radicals (23). The combination of ethanol extract from tapak dara leaves and ethanol extract from galangal rhizomes in a ratio of 2:1 showed an increase in antioxidant activity. This increase is believed to be the result of the corresponding chemicals in each sample interacting with each other. This interaction can produce synergy between these compounds,

increasing their ability to fight free radicals and overall antioxidant activity (24). The test results indicate that additional molecules may influence the anticipated reactions in addition to the active chemicals that serve as the main components of the plant (25). According to (26)The flavonoids contained in the extract/fraction samples which may still be glycoside flavonoids can contribute to a decrease in antioxidant activity.

Differences in IC50 values for each extract or combination of extracts are caused by variations in the distribution of the type and number of secondary metabolite groups that act as antioxidants, which are influenced by the polarity of the solvent used in the extraction (27). Ethanol was chosen as the solvent in this study because ethanol has better solubility in DPPH, even though methanol has a higher polarity (16). This is in accordance with research which shows that ethanol extract is better at removing free radicals than water extract (28). The interaction between these two types of extracts, each of which contains secondary metabolites, occurs when combined. This interaction can potentially strengthen the effect at low concentrations or vice versa, namely mutually weakening. It is important to remember that the antioxidant response does not always increase with higher concentrations. The antioxidant response is not necessarily better at higher concentrations. Therefore, careful consideration needs to be taken in determining the optimal concentration of extract combinations to achieve the desired effect (29).

CONCLUSIONS

Significant antioxidant activity was demonstrated when kencur rhizomes and tapak dara leaves were combined in ethanol extract. Total flavonoid levels in a combination with a ratio of 2:1 were higher than the single extract. Ethanol extract of kencur rhizome and tapak dara leaves in a ratio of 2:1 with a value of 9.15 ppm showed the highest antioxidant activity. This value is categorized as very strong, indicating that this combination has high potential in fighting free radicals and as an antioxidant agent.

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