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The Screening of Antioxidant Activities of *Meniran* Plants (*Phyllanthus niruri* Linn) and Guava Leaves (*Psidium guajava* Linn) Combination Using DPPH Free Radical Method

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ABSTRACT

Degenerative disease is a serious health problem and causes a lot of deaths in Indonesia. One of the most dangerous degenerative diseases is cancer. Free radicals play a role in oxidative stress in the later stages of carcinogenesis. Antioxidant delays or inhibits cellular damage mainly through its free radical scavenging property. *Meniran* plants and guava leaves have high radical-scavenging activities. The previous studies reported that the combination of ginger and *Meniran* plant extract has a stronger antioxidant activity than the extract of a single plant. This research aims at determining the antioxidant activities of *Meniran* plants and guava leaves compared with its singular form. This research was conducted from October to December 2016 at the Chemical Laboratory of Nasional Health Science Institute and the Center for Development and Research of Medicinal Plants and Traditional Medicine, Tawangmangu, Karanganyar. The antioxidant activity assay was done using DPPH free radical method and vitamin C was used as the control. They were measured with UV-Vis Spectrophotometer. This study concludes that the IC50 value of *Meniran* plants was 30.689 ppm and the IC50 value of guava leaves was 13.7859. The IC50 values of *Meniran* plant and guava leaf combination with various ratios were 20.6095 ppm (1:1), 12.5629 ppm (1:2), and 16.841 ppm (2:1). The combination of *Meniran* plant and guava leaf extract (1: 2) had the strongest antioxidant activity of 12.56 ppm.

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INTRODUCTION

Degenerative disease is a severe health problem that contributes to the many deaths in Indonesia. One of the most dangerous degenerative diseases is cancer. Lung, liver, stomach, colorectal, and breast cancers are the major causes of death every year in Indonesia. Free radicals have established a role for oxidative stress in the later stages of carcinogenesis. (Guyton & Kensler, 1993). Free radicals are generated by our body by various endogenous systems and exposure to different physiochemical conditions or pathological states.

Proteins, lipids, carbohydrates, and nucleic acids are susceptible to oxidative damage as the result of a free radical attack. A molecule is stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing the capacity of free radical to damage the vital cell molecule. Antioxidant delays or inhibits cellular damage mainly through its free radical scavenging property (Halliwell, 1995).

These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital cell molecules are damaged. Some of the antioxidants produced during normal metabolism in the body are glutathione, uric acid, and ubiquinol (Shi et al., 1999). The body cannot produce the micronutrients such as vitamin E (α-tocopherol), vitamin C (ascorbic acid), and B-carotene, so they must be supplied from diet or rich antioxidant plants such as *Meniran* plants and guava leaves.

Those plants are commonly used in foods as well as cosmetics for their potent antioxidant activities. *Meniran* plants contain phytochemical components such as flavonoids, alkaloids, steroids, and tannins (Wulandari, 2011). These compounds function as antioxidants. *Meniran* plants exhibit high radical-scavenging activities (IC50 = 7.50 ppm) (Sasidharan et al., 2007). Guava leaves also exhibit high radical-scavenging activities (IC50 = 37.14 ppm) (Maulana et al., 2016). The phytochemical compounds found in guava leaves are saponins, steroids, and triterpenoids (Arya et al., 2012).

Wulandari (2011) reported that a combination of ginger and *Meniran* plant extract has a stronger antioxidant activity than the singular form. Based on the explanation, the researchers are triggered to research the screening of antioxidant activities of Meniran plants (*Phyllanthus niruri* Linn) and guava leaves (*Psidium guajava* Linn) combination by using DPPH free radical method. This method is used to determine the antioxidant activities of *Meniran* plants (*Phyllanthus niruri* Linn) and guava leaves (*Psidium guajava* Linn) combination compared to the antioxidant activities of a single material and decide the material with stronger antioxidant activities.

MATERIALS AND METHODS

Time and places

The research was conducted from October to December 2016 at the Chemical Laboratory of Nasional Health Science Institute and the Center for Development and Research of Medicinal Plants and Traditional Medicine, Tawangmangu, Karanganyar.

Instruments

The instruments used in this research were a UV-visible AE lab S80 spectrophotometer, cuvette, filler pipette, filter paper, rotary evaporator, flask glass, beakers glass, Erlenmeyer glass, stir bar, chamber glass, vial bottle, pushball, aluminum voile, and vortex.

Materials

Meniran plants and guava leaves were collected from our garden in Serengan Sub-district, Surakarta. Amyl alcohol, Wagner's reagent, Mayer's reagent, Dragendorf's reagent, lead acetate, chloroform, concentrated sulphuric acid, $FeCl_3$ 1%, ether, concentrated H_2SO_4, DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate), and vitamin C.

Procedures

The collection of materials

Approximately 1 kg of round rod-shaped fresh green *Meniran* plants and fresh guava leaves were collected. Those plants were washed thoroughly 3-5 times with tap water then dried in an oven with a temperature of 40ºC. After drying was completed, the materials were ground in a mixer and the powder was kept in small plastic bags with paper labeling. (Arlofa, 2015).

Extraction

250 grams dried *Meniran* plants and guava leaves were exhaustively extracted with 96% ethanol and evaporated to dryness under reduced pressure. Aliquots of the extracts were solubilized in 96% ethanol to a final maceration with a concentration of 1000 ppm.

Phytochemical screening

Preliminary qualitative phytochemical screening was performed with the following methods:

Flavonoids: 2 mL of extract was added to 0.1 mg amyl alcohol and 0.4 ml of amyl alcohol. The formation of red or orange rings indicated the presence of flavonoids (Arlofa, 2015).

Alkaloids: 0.5 mL of extract was mixed with 5 mL of Dragendorf's reagent. The appearance of red precipitate indicated the presence of alkaloids (Le Quesne). 0.5 ml of extract was mixed with 5 ml of Mayer's reagent. The appearance of yellow precipitate indicated the presence of alkaloids (vanilla). 0.5 mL of extract was mixed with 5 mL of Wagner's reagent. The appearance of brown precipitate indicated the presence of alkaloids (Lingappa & Lingappa, 1967).

Tannins: 2 mL of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins (Treare & Evans, 1985)

Saponins: 5 mL of extract was mixed with 20 mL of distilled water and then agitated in a graduated cylinder for 15 minutes. The formation of foam specified the presence of saponins (Kumar, 2009).

Steroids: 1 mL of the extract was dissolved in 10 ml of chloroform and an equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turned red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (Gibbs, 1974).

Triterpenoids: 5 mL of the extract was dissolved in 5 mL of chloroform and 5 drops of concentration H_2SO_4 were added by sides of the test tube. The upper layer turned yellow, indicating the presence of triterpenoids (Arya et al., 2012).

DPPH stock solution

As much as 10.0 mg DPPH was dissolved in 100 mL of ethanol 96% (100 ppm). The solution was stored at low temperature and shielded from sunlight (Sumiyani, 2007).

DPPH standard solution

DPPH stock solutions (100 PPM) were diluted to final concentrations of 5, 10, 20, 25, and 30 ppm in ethanol (Sumiyani, 2007).

Maximum wavelength determination.

3.0 mL of 40 ppm DPPH solution was added with a 1.5 mL sample. The absorbance value was measured at the wavelength (λ) of 400-700 nm (Sumiyani, 2007).

Operating time determination.

3.0 mL of 40 ppm DPPH solution was added with a 1.5 mL sample. The absorbance value was measured at maximum wavelength until it showed a constant absorbance value.

***Meniran* plant extract spectrophotometric assay**

Meniran plant stock solutions: *Meniran* plant extract weighed as much as 20.0 mg was dissolved to 100.0 mL of ethanol (200 ppm). These plant stock solutions were diluted to the final concentrations of 5, 10, 15, 20, 25, 30 ppm. 3.0 mL of DPPH 40 ppm was added to a 1.5 mL sample of each concentration. After 30 minutes, the absorbance values were measured.

Guava leaf extract spectrophotometric assay

Guava leaf stock solutions: Guava leaf extract weighed as much as 20.0 mg was dissolved to 100.0 mL of ethanol (200 ppm). Guava leaf stock solutions were diluted to the final concentration of 5, 10, 15, 20, 25, 30 ppm. 3.0 mL of DPPH 40 ppm was added to a 1.5 mL sample of each concentration. After 30 minutes, the absorbance values were measured.

***Meniran* plant and guava leaf extract combination spectrophotometric assay**

The mixtures of each combination from *Meniran* plants and guava leaves were made with different volume ratios as shown in Table 1. 3.0 mL of DPPH 40 ppm was added to a 1.5 mL sample of each combination. After 30 minutes, the absorbance values were measured.

Table 1. Volume ratio for each combination

Combination	200 ppm *Meniran* extract (mL)	200 ppm Guava leaf extract (mL)	Vol. (mL)	Concentration (ppm)
1:1	12.5	12.5	50	100
1:2	8.3	16.7	50	100
2:1	16.7	8.3	50	100

Control solution spectrophotometric assay

1.5 mL of 96% ethanol was added with 3.0 mL of 40 ppm DPPH solution, and after 30 minutes, the absorbance value was measured.

Vitamin C spectrophotometric assay

Vitamin C stock solutions: Vitamin C weighed as much as 10.0 mg was dissolved to 100.0 mL of ethanol 96% (100 ppm). Vitamin C stock solutions were diluted to the final concentrations of 2, 3, 4, and 5 ppm. 3.0 mL of DPPH 40 ppm was added to a 1.5 mL sample of each concentration. After 30 minutes, the absorbance values were measured.

RESULT AND DISCUSSION

Maceration was the extraction method used in this research. The ethanol extract yields of *Meniran* plants and guava leaves were obtained and calculated as the results presented in Table 2.

A quantitative analysis using radical scavenging DPPH assay refers to Von Gadow's research in 1997. The ethanol extract yield of *Meniran* plants in this research was higher than the extraction yield reported in the previous study by Wulandari, which was 7.04 % (Wulandari, 2011). The ethanol extract yield from guava leaves also had a higher value in this research than reported in the study by Hidayati, which was 18.25 % (Hidayati, 2015). Proper control of injection rate and pressure in boiler-operated units is necessary to optimize the temperature of extraction for maximal yield (Singh, 2008). Phytochemical components such as alkaloids, flavonoids, saponins, tannins, and steroids were determined, as presented in Table 3. The phytochemical screening experiments show that *Meniran* plants and guava leaves contain the phytochemical components. The Guava leaves had more phytochemical components such as triterpenoids. It is the same as the conclusion of the previous studies (Maulana et al., 2016) and (Arya et al., 2012)

Table 2. Summary of Meniran Plant and Guava Leaf Extract Yields

Material	Weight (g)	Simplicia Weight (g)	Yield (by f.wt) (%)
Meniran plants	250	29	11.6 %
Guava leaves	250	51	20.4 %

Table 3. Phytocemical Screening Results

No	Test	*Meniran* Plants	Guava Leaves
1.	Alkaloids:		
	a. Dragendorf	positive	positive
	b. Mayer	positive	positive
	c. Wagner	positive	positive
2.	Flavonoids	positive	positive
3.	Saponins	positive	positive
4.	Tannins	positive	positive
5.	Steroids	positive	positive
6.	Triterpenoids	negative	positive

Fig. 1 shows the graph of the linear regression equation of y = 4.4297x + 21.691. X was the slope and it had 21,691 as the intercepts. It can be seen that the IC50 value of vitamin C was 6.390726. It had a very strong antioxidant activity (Andriani et al., 2019). Fig. 2 demonstrates that the singular form of guava leaves had a stronger antioxidant activity than *Meniran* plants. It can be seen from the IC50 value of guava leaves that was lower than the IC50 of the combination of guava leaves and *Meniran* plant. The lower the IC50 value is, the stronger antioxidant activity will be (Harningsih & Wimpy, 2018)

The results of the analysis also depict the differences in the IC50 values in all combinations. The combination of *Meniran* plants and guava leaves with the ratio of 1: 2 had the strongest IC50 value when compared with the singular form and the other combinations because the guava leaves had more phytochemical components such as triterpenoids (Arya et al., 2012) than *Meniran* plants. The report of a previous study concluded that triterpenoids have stronger scavenging activity to DPPH than the other phytochemical components (Cipak et al., 2006). When a solution of DPPH free radical is mixed with an antioxidant or reducing component, the color turns from purple to yellow of the corresponding hydrazine (Fig 3). However, the reactional mechanism between the antioxidant and DPPH depends on the structural conformation of the antioxidant as presented in Fig. 3 (Bondet et al., 1997)

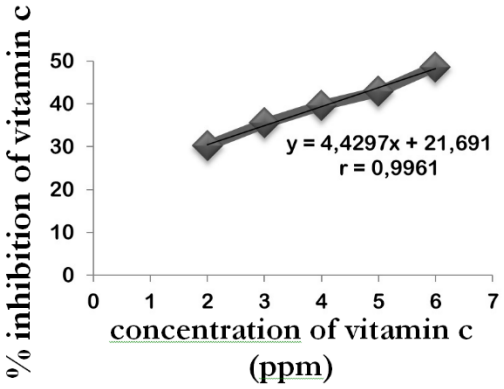

Fig 1. % Inhibition of vitamin C

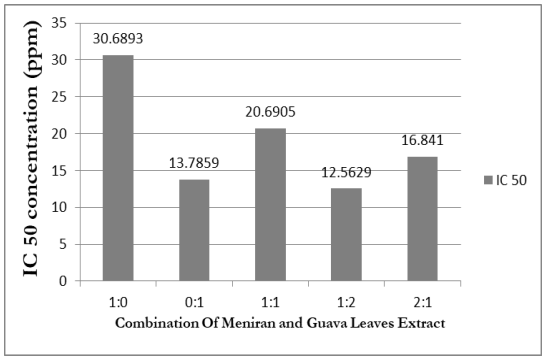

Fig 2. IC50 graph of *Meniran* plants and guava leaves singular form and the combination

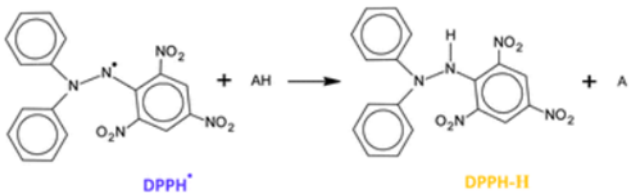

Fig. 3. DPPH Reaction (Pyrzynska & Pękal, 2013)

CONCLUSION

The phytochemical components were found in *Meniran* plant and guava leaf extract are alkaloids, flavonoids, saponins, tannins, steroids. Guava leaves contain more phytochemical components such as triterpenoids. The antioxidant activity scavenging against DPPH from the combination of ethanol extracts and Meniran plants (*Phyllanthus niruri* L.) and guava leaves (*Psidium guajava* L.) analyzed using UV-Vis spectrophotometry showed that *Meniran* plant and guava leaF combination (1:2) has the strongest antioxidant activity among the other combinations and singular form.

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