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Antioxidant activity of extracted *Phyllanthus niruri* L.) , *Sauropus androgynus* (L.) Merr.) and Its Combination Used DPPH with Spectrophotometric UV-Vis

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ABSTRACT

Background: Meniran herb and katuk leaves are herbal plants native to Indonesia and are known to have flavonoid compounds which have antioxidant activity to inhibit free radicals. This study aims to determine whether or not there is an increase in the antioxidant activity of herbal extracts of meniran and katuk leaves either singly or in combination using the DPPH method by UV-Vis Spectrophotometry.

Methods: This study was an experimental study using 100 grams of Meniran herb (Phyllanthus niruri L.) and Katuk leaves (Sauropus androgynus (L.) Merr.) each which were macerated with ethanol solvent and then made thick extracts by evaporated them in a rotary evaporator. After that single extract, combination extract (1:1; 1:2; 2:1) and standard quercetin as a reference were made in 5 concentration variations, namely 10 ppm, 8 ppm, 6 ppm, 4 ppm, and 2 ppm. After obtaining the silencer, the IC50 value is calculated as a parameter for measuring antioxidant activity.

Results: The results showed that the IC50 value of Meniran Herb Extract (Phyllanthus niruri L.) was 10,38 ppm, Katuk Leaf was 17.63 ppm, Combination Extract (1:1) was 10,09 ppm, Combination Extract (1:2) was 15,05 ppm, Combination Extract (2:1) was 7,36 ppm, and the IC50 value of Quercetin as a standard material for comparison is 2,65 ppm.

Conclusion: This study showed that the antioxidant activity of the combination of Meniran Herb Extract and Katuk Leaf Extract is better than both single extracts with a very strong combination obtained from a ratio of 2:1.

Keywords: Antioxidants, DPPH, Meniran Herbs, Katuk Leaves, Combination extracted

I. INTRODUCTION

Indonesian herbs are medicinal plants that grow and are cultivated in Indonesia and used for health purposes. One of them is the herb meniran (Phyllanthus niruri L.). Meniran has various properties, including hepatoprotector, diuretic, and treatment of kidney stones (nephrolithiasis) (Kemenkes RI, 2016). In the pharmaceutical industry, the use of meniran as an immunomodulator is also proven by a patented phytopharmaceutical product called Stimuno® (BPOM, 2020). Several scientific studies that have been conducted both in vivo and in vitro show that meniran herb has pharmacological potential in various conditions, namely as an antiviral (Hepatitis B antiviral), antidiabetic and antioxidant marked by high flavonoid content (Lee et al., 2016). The flavonoid that has been successfully identified from meniran herb is quercetin, which is known to be important in nutrition because of its ability as an antioxidant (Rusmana et al., 2017).

Beside meniran, another plant that is native to Indonesia is katuk (Sauropus androgynus (L.) Merr.). Empirically, katuk leaves are known because they are commonly consumed as cheap but nutritious vegetables and are known for their benefits as breast milk enhancers, fever-reducing drugs and cough medicines in various regions (Hayati, 2016). In addition, katuk leaves are identified as a source of natural materials that are potentially rich in antioxidants because they contain flavonoids (Andarwulan et al., 2010). Flavonoids have great potential to fight diseases caused by free radicals (Sayuti and Yenrina, 2015).

Antioxidants are compounds that have the potential to neutralise and prevent damage and are able to protect the body from the dangers of free radicals. Free radicals are molecules that react easily and tend to be unstable due to the loss of their electron pair. This can lead to cell damage and degenerative diseases such as cancer, autoimmunity, stroke, heart disease, and hypertension. Although the body also produces antioxidants, additional antioxidant consumption is

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needed as an antidote to free radicals that cause degenerative diseases (Werdhasari, 2014).

The DPPH or 1,1-Diphenyl-2-picrylhydrazyl method is a method used in the determination of antioxidant activity. Antioxidant activity in samples tested using the DPPH method is indicated by the IC50 value, which is defined as the concentration required to reduce DPPH free radicals by 50% (Munteanu and Apetrei 2021)

In a previous study, the results stated that meniran herb extract (Phyllanthus niruri L.) had an IC50 value of 17.59 ppm categorized as a very strong antioxidant. Phytochemical screening of meniran herb extracts showed flavonoids, terpenoids, tannins and saponins (Tambunan, Swandiny and Zaidan, 2019). In addition, Hikmawanti et al, (2021) stated that katuk leaf extract (Sauropus androgynus (L.) Merr.) produced an IC50 value of 9.65 ppm categorized as a strong antioxidant and contained flavonoids, alkaloids, tannins, saponins and steroid compound

The antioxidant activity of meniran herb and katuk leaf has been studied singly and is known to have flavonoid compounds that have antioxidant activity. However, the utilization of the two plants when combined is not yet known. This is the reason the author wants to examine the measurement of antioxidant activity of both plant extracts, namely meniran herb extract (Phyllanthus niruri L.) and katuk leaf extract (Sauropus androgynus (L.) Merr.) when combined. It is hoped that this research can be utilized.

II. METHODS

This type of research is an experiment by measuring the antioxidant activity contained in meniran herb extract *(Phyllanthus niruri* L.), katuk leaf extract *(Sauropus androgynus* (L.) Merr.) singly and in combination using the DPPH method using UV-Vis spectrophotometry.

Tools and Materials

The tools used are 10 ml volume pipettes, UV-Vis spectrophotometry, vials, chemical reaction tubes, cuvettes, knives, brown maceration bottles, blenders, rotary evaporators, analytical balances, funnels, measuring *cups* , measuring flasks, stir sticks, cups, flannel cloth, dropper pipette, dropper plate and aluminum foil. The materials used are Meniran herb *(Phyllanthus niruri* L.), Katuk leaves *(Sauropus androgynus* (L.) Merr.), 96% ethanol solvent, DPPH reagent, quercetin, Mg metal, concentrated HCl reagent, Mayer's reagent, Lieberman Buchard's reagent, distilled water, H2SO4 2N and FeCl3

a) Flavonoid

Pipette 1 ml of meniran herbal extract/katuk leaf extract, put it in a test tube, add 2 ml of 2NH2SO4 and 2 drops of Mayer's reagent then shake. Test positive for alkaloids if a yellow or white precipitate forms.

b) Saponin

Pipette 1 ml of meniran herbal extract/katuk leaf extract, put it in a test tube, add 2 ml of distilled water, then shake vigorously for 1 minute. The presence of stable foam within 10 minutes indicates positive saponin.

c) Tannin

Pipette 1 ml of meniran herb extract/katuk leaf extract, put it in a test tube, add 2 drops of FeCl3,if a green/blackish blue color forms, it indicates positive tannin.

d) Terpenoids-Steroids

Pipette 2 ml of meniran herb extract/katuk leaf extract, put it in a test tube, add 0.5 ml of chloroform then filter, then place the filtrate in a drop plate, add Lieberman Burchard's reagent or 2 drops of acetic acid (CH3COOH) and 2 drops of acid . concentrated sulfate (H2SO4) . If a brownish red color forms, it is positive for terpenoids. If a greenish blue color forms, it is positive for steroids.

Preparation of 40 ppm DPPH Solution

A total of 4 mg of DPPH powder was weighed and put into a 100 ml measuring flask, then dissolved by adding 96% ethanol solvent up to the mark to obtain a concentration of 40 ppm or 0.004% (Antarti and Lisnasari, 2018).

Antioxidant Activity Test

a) Determination of the Maximum Wavelength of DPPH Solution

A total of 4.0 mL of 40 ppm DPPH solution was pipetted into a cuvette and measured using a UV-Vis spectrophotometer with a wavelength of 400-600 nm and then the absorbance at the maximum wavelength was recorded (Tahir, Suhaenah and Rahim, 2020).

b) Measurement of Antioxidant Activity of Meniran Herbal Extract, Katuk Leaf Extract, Extract Combination, Quercetin Comparative Standard

Weigh the herbal extracts of meniran, katuk leaves and quercetin respectively

a total of 50 mg as well as a combination extract with a ratio of 1:1; 1:2; 2:1 and dissolved in 50 ml of ethanol to a concentration of 1000 ppm, diluted to 100 ppm by pipetting 10 ml of the stock solution

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and adding 100 ml of ethanol. Then vary the concentration of the solution, namely 2 ppm, 4 ppm, 6 ppm, 8ppm, and 10 ppm.
From the sample solution, 2 ml of each was pipetted and put into vials, then pipetted 2 ml of 2 ml of DPPH40 ppm standard solution (volume ratio 1:1). The mixture in a vial covered with aluminum foil was left for 30 minutes, then absorbance was measured at a maximum wavelength of 516 nm using UV-Vis spectrophotometry (Indra, Nurmalasari and Kusmiati, 2019).

Determination of *IC50 Value*

Determination of free radical scavenging activity was carried out using the DPPH method. Theresults of the free radical scavenging activity of the ethanol extract of meniran herb and katuk leaves singly and in combination will be compared with quercetin as positive control. The percentage of damping obtained by each sample is then substituted on the x and y axes in the linear regression equation. This equation is used to obtain the IC50 value with the formula Y = bX + a, where the Y value is 50 and the X that will be obtained is the IC50 value (Indra, Nurmalasari and Kusmiati, 2019

III. RESULTS AND DISCUSSION

Results

Maceration Extraction

From extraction of 100 grams of each sample, 30.05 grams of Meniran Herb thick extract and 36.77 gram of Katuk Leaf thick extract were obtained. So that the yields of Meniran Herb and Katuk Leaf extracts were 30.05% and 36.77% respectively.

Identify Chemical Ingredients

The results of identifying the chemical content of meniran herb extract and katuk leaves canbe seen in table 1 and table 2.

Tabel 1. Identification of the Chemical Content of Meniran Herb

Content	Reagent	Result as Positive	Observation
Flavonoid	HCl pt Logam Mg	Redness	(+)
Alkaloid	H_2SO_4 2N Pereaksi Mayer	Yellow with precipitate	(-)
Tanin	$FeCl_3$	Blue Dark	(+)
Saponin	Aquadest, kocok	Foam Stable	(+)
Terpenoid	Kloroform Lieberman Burchard	Brown redness	(+)
Steroid	Kloroform Lieberman Burchard	Green Blue	(-)

Table 2 Identification of the Chemical Content of Katuk Herb

Content	Reagent	Result of	Positive Observation
Flavonoid	HCl p Magnesium	Redness	(+)
Alkaloid	H_2SO_4 2N Reagent Mayer	Yellow with precipitated	(+)
Tanin	$FeCl_3$	Blue Dark	(+)
Saponin	Aquadest, Shake	Foam Stable	(+)
Terpenoid	Kloroform Lieberman Burchard	Brown redness	(-)
Steroid	Kloroform Lieberman Burchard	Green Blue	(+)

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Testing Activity Antioxidant

Serial Concentration of DPPH dilution to have absorbance maximum of DPPH 40 ppm

Figure 1

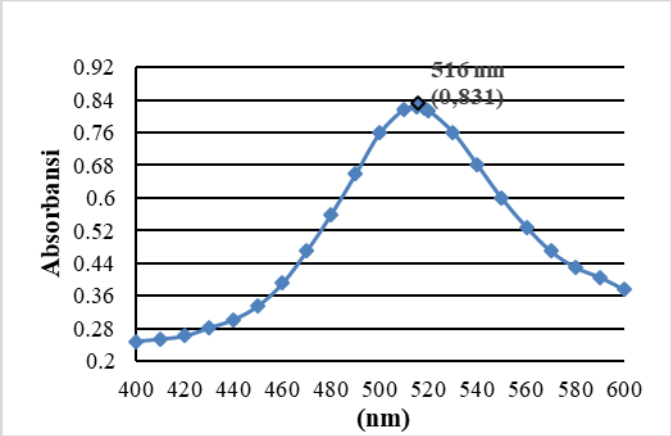

Figure 1. Absorban Maximum of DPPH 40 ppm 516 nm

Tabel 3. Activity of Antioxsidant Exstract Herb Meniran dan Leaf Katuk

Sampel	Time Minute	PPM	% IC50
Exstract Herb Meniran	30 minute	10 ppm	52,10%
		8 ppm	44,28%
		6 ppm	42,71%
		4 ppm	40,91%
		2 ppm	40,19%
Extract Katuk Leaf	30 minute	10 ppm	44,16%
		8 ppm	42,23%
		6 ppm	41,87%
		4 ppm	38,62%
		2 ppm	38,26%
Combination Extract Herb Meniran and Katuk Leaf (1:1)	30 minute	10 ppm	50,42%
		8 ppm	47,29%
		6 ppm	45,24%
		4 ppm	42,47%
		2 ppm	41,63%
Combination Extract Herb Meniran and Katuk Leaf (1:2)	30 minute	10 ppm	45,84%
		8 ppm	42,59%
		6 ppm	42,47%
		4 ppm	40,19%
		2 ppm	38,02%
Combination i Extract Herb Meniran and Katuk Leaf (2:1)	30 minute	10 ppm	59,56%
		8 ppm	49,45%
		6 ppm	43,08%
		4 ppm	41,87%
		2 ppm	40,19%
Quercetin	30 minute	10 ppm	68,47%
		8 ppm	66,04%
		6 ppm	54,75%
		4 ppm	52,10%

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	2 ppm	50,42%

Tabel 4. IC_{50} Value 0f Extract Herb Meniran, Extract Katuk, Extract Combination and standard of Quercetin

Sample	Time Minute	Formula Regression	IC_{50}
Extract Herb Meniran	30 Minute	y = 1,3598x + 35,884	10,38 ppm
Extract Katuk Leaf	30 Minute	y = 0,7702x+ 36,414	17,63 ppm
Combination Extract Herb Meniran dan Leaf Katuk (1:1)	30 Minute	y = 1,1191x + 38,7	10,09 ppm
Combination Extract Herb Meniran dan Katuk Leaf (1:2)	30 Minute	y = 0,9025x + 36,414	15,05 ppm
Combination Extract Herb Meniran dan Katuk Leaf (2:1)	30 Minute	y = 2,3165x + 32,936	7,36 ppm
Quercetin	30 Minute	y = 2,503x + 43,345	2,65 ppm

IV. DISCUSSION

In this study, researchers tested the antioxidant activity in single and compare to a combination of two types of plants extracted, namely Meniran Herb (*Phyllanthus niruri* L.) and Katuk Leaf (*Sauropus androgynus* (L.) Merr.). Looking for if any different in single and combination of antioxidant activity. The antioxidant compounds contained in meniran herbs and katuk leavesare flavonoids. This research was conducted to determine the amount of antioxidant activity contained in the combined extract of Meniran Herbal Extract and Katuk Leaf Extract using the DPPH method using UV-Vis spectrophotometry. Seems any different in single activity and combination.

Antioxidant activity is expressed in the IC50 value of a sample. The lower the IC50value, the stronger it is in reducing free radicals so that the antioxidant activity is higher (Molyneux, 2004)

Meniran herb is a herbal medicinal plant which has properties as an immune booster, diuretic, kidney stone treatment, antidiabetic, antipyretic and anti-inflammatory (Ministry of Health of the Republic of Indonesia, 2016; Lee et al, 2016) . Based on research that has been carried out, the IC50 value of Meniran Herb is obtained at 10.38 ppm. Meanwhile, quercetin as a comparison standard has an IC50 value of 2.65 ppm. This shows that the antioxidant activity of meniran and quercetin herbs is included in the very strong antioxidant category. However, the IC50 value of quercetin is lower, which means that the antioxidant activity of quercetin is stronger than that of meniran herb. Thisis because quercetin is a pure antioxidant compound, while the meniran herb extract is still in the form of a mixture of other compounds (Handayani, Kurniawati and Rasyid, 2020). The results of the antioxidant activity of meniran herb with an IC50 value of 10.38 ppm are in the very strong category in accordance with research conducted by (Tambunan, Swandiny and stated that the meniran herb has an IC50 value of 17.59 ppm, which is included in the very strong antioxidant category. Katuk leaves are included in herbal medicines that are used as a breast milk enhancer, fever reducing medicine, cough medicine and antibacterial (Santoso, 2014). Based on research that has been carried out, the IC50 value for Katuk leaves is 17.63 ppm. Meanwhile, quercetin as a comparison standard has an IC50 value of 2.65 ppm. This shows that the antioxidant activity of katuk leaves and quercetin is included in the very strong antioxidant category. However, the IC50 value of quercetin is lower, which means that the antioxidant activity of quercetin is stronger than that of katuk leaves. This is because quercetin is a pure antioxidant compound, while katuk leaf extract is still in the form of a mixture of other compounds (Handayani, Kurniawati and Rasyid, 2020). The antioxidant activity of katuk leaves with an IC value of 5017.63 ppm in the very strong category has a lower IC50 value compared to research conducted with different concentrations by Hikmawanti et al, (2021) which states that the IC50 value of Katuk leaves is 90.65 ppm which is included in the category powerful antioxidant.

Based on table 4, the herbal extracts of meniran and katuk leaves, singly or in combination, have antioxidant activity that is different from one another. Differences in IC50 values can occur due to the different amounts of content and interactions of secondary metabolite compounds in each extract (Rudiana, Inriatmoko, and Komariah, 2021). However, meniran and katuk leaf herbal extracts singly or in combination are still included in the very strong antioxidant

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category because they have an IC50 value of less than 50 ppm. So the two extracts will produce better antioxidant activity when combined. In this study, the combination of meniran herbal extract and katuk leaves in a ratio of 2:1 had the lowest IC50 value, namely 7.36 ppm compared to other extract combinations. This is in accordance with the results of research by Harningsih and Wimpy (2018) who used a combination of Kersen Leaf and Soursop Leaf extracts in a 2:1 ratio produces an IC50 value of 6.91 ppm, which means it is included in the very strongantioxidant category.

V. CONCLUSION

Based on the results of the research it can be concluded that the IC50 value of Meniran Herbal Extract *(Phyllanthus niruri* L.) and Katuk *(Sauropus androgynus* (L.) Merr.) Leaf Extract is better to used in combination made in a 2:1 ratio to have of 7.36 ppm with quercetin as a comparison standard of 2.65 ppm.

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