Making Formulation of Tea Bags Combination of Kecibeling Leaves (*Strobilanthes crispa* (L.) Blume), Salam Leaves (*Syzygium polyanthum* (Wight.) Walp.) and Meniran Herbs (*Phyllanthus niruri* L.) As Antioxidants

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ABSTRACT

The more incessantly the motto "*back to nature*" various types of plants in Indonesia are used by the community as traditional medicine. Kecibeling plants, salam and meniran herbs are one of the traditional medicinal ingredients that are often used. The potential development of the combination of kecibeling leaves, bay leaves and meniran herbs was chosen as a tea bag preparation because it is simple, cheap, and practical. The purpose of this study was to formulate a combination of kecibeling leaves, salam and meniran herbs as an antioxidant. This research was conducted by collecting fresh leaves of the kecibeling plant, salam and meniran herbs from Pematangsiantar, then used as simplicia and then formulated in teabags with varying weights. Then the formula is tested for characteristics which include water content test, pH test of the preparation, organoleptic test including hedonic test and antioxidant activity test.The results of testing the water content in each formula are 3.72%; 5.07% and 6.81%. The results of the pH test of teabag preparations from each formula were 6.7-7.Based on the results of the Hedonic test, Formula II with a weight of 0.95g was preferred by the panelists, so it was continued to be tested for activity as an antioxidant. The results of the antioxidant activity test showed that in Formula II (0.95 g) has antioxidant activity with the category "Medium".

Keywords: antioxidant activity; characteristics; combination; simplicia; tea bag

INTRODUCTION

Free radicals are atoms or molecules that contain an unpaired electron in their outermost orbital. Free radicals are unstable and highly reactive, i.e. they tend to react with other molecules to achieve stability. Radicals with high reactivity can start a chain reaction in one formation, giving rise to abnormal compounds and starting a chain reaction that can damage important cells in the body (Badarinath *et al*., 2010).

Antioxidants are electron donating compounds. Antioxidants work by

donating one electron to compounds that are oxidants so that the activity of these oxidant compounds can be inhibited. Antioxidants stabilize free radicals by complementing the electron deficiency of free radicals, and inhibit the chain reaction of free radical formation (Liberty, 2012).

The search for sources of antioxidants is more directed at natural antioxidants, especially those from plants. Natural antioxidants have a better level of safety. Flavonoids are one of the antioxidants that are widely distributed in plants (Winarsi, 2007).

Kecibeling (*Strobilanthes crispus* (L.) Blume) is a type of plant that is generally planted by the community as a hedge (Dalimartha, 2007). Kecibeling leaves (*Strobilanthes crispus* (L.) Blume) contain large amounts of active compounds such as minerals (potassium, calcium, sodium, potassium, iron and phosphorus), water-soluble vitamins (C, B1, and B2), vitamin E, catechins, tannins.
, coumarins, flavonoids, and steroids (Setyaningsih, 2008).

Salam leaves have been studied to contain flavonoids which have antioxidant activity (Agus and Agustin, 2008). Flavonoids can improve endothelial function. This effect is an advantage of flavonoids on the risk of cardiovascular disease (Agus and Agustin, 2008).

Salam leaves is a plant that is often found in Indonesia. Plants that have a Latin name (*Syzygium polyanthum* (Wight.) Walp.) as a medicinal plant native to Indonesia are widely used by the public to reduce cholesterol, diabetes, hypertension, gastritis, and diarrhea.

Salam leaves are known to contain flavonoids, selenium, vitamin A, and vitamin E which function as antioxidants (Riansari, 2008). Bark, roots and leaves can be used as a remedy for itching. And the wood is used for building materials (Sembiring, Winarti and Baringbing, 2008).

Meniran (*Phyllanthus niruri* L.) is a wild plant of the *Euphorbiaceae* tribe that lives in tropical climates. In Indonesia this plant is very easy to find on the edge of the road, vacant land, gardens, rivers and even in the yard of the house. The results of research on the chemical content of meniran herbs showed that there were chemical compounds of essential oils, flavonoids, alkaloids, arbutin, glycosides, anthraquinones, phenolic compounds, and tannins (Sudarsono *et al*., 1996).

The potential development of the combination of kecibeling leaves, salam leaves and meniran herbs was chosen to be a tea bag preparation, because of the advantages of the method of making teabag preparations which are simple, inexpensive, and practical. Currently, the preferred form of tea preparation is tea bags, because of its practical preparation. This is also interesting and unique, because this tea is not made from tea leaves but from kecibeling leaves, salam leaves and meniran herbs.

MATERIAL AND METHODS

Kecibeling leaves (*Strobilantes crispa* (L.) Blume), salam leaves (*Syzygium polyanthum* (Wight.) Walp.), meniran herbs (*Phyllanthus niruri* L.), pH 4 solution, pH 7 solution, methanol, distilled water, silica gel and *2 2-diphenyl-1-picrylhydrazyl* (DPPH), *Blender* (Miyako), Desiccator, Impluse Sealer Q2, Drying cabinet, pH meter (Lutron PH-201), Shimadzu UV-Vis 1800 Spectrophotometer, Digital scale (AND HT-120*).

Procedures

Simple material processing

The plants used were kecibeling leaves, Salam leaves and meniran herbs taken in the Pemangsiantar area (Figure 1). A total of 1 kg of each plant was processed into simplicia with several stages, namely sampling, wet sorting,

washing, chopping, drying, dry sorting and refining (Directorate General of POM, 1989) at a temperature of 45-60 °C for ±3 days in a drying cabinet. The results of plant simplicia can be seen in (Figure 2).


Kecibeling leaves


Salam leaves


Meniran herb

Figure 1. Plant pictures

Kecibeling leaves simplicia

Salam leaves simplicia

Meniran herb simplicia

Figure 2. Simple picture

Water content test

Testing the water content by means of the dish in an oven for 15 minutes at a temperature of 105℃, then the cup is cooled in a desiccator for 15 minutes and weighed. The formula was weighed and put in a dry cup, then baked at a temperature of 105℃ for 5 hours, cooled in a desiccator for 15 minutes and weighed (B1). After that the cup + sample was dried again in the oven for ± 30 minutes at a temperature of 105℃ to constant weight, then cooled again in a desiccator for 15 minutes and weighed (B2) (AOAC, 2012).

$$\% \text{ water content} = \frac{W1-W2}{W1} \; x \; 100\%$$

Preparation of pH test

Measurement of pH was carried out on formulations I, II, and III using a pH meter. The pH meter was calibrated using

a phosphate buffer solution at pH 4 and 7. Washed the electrodes with water and then dried and then dipped the electrodes into each formula in turn, then waited a few minutes until the results showed a constant pH (SNI 6986-11, 2019).

Organoleptic testing

Parameters for testing the organoleptic characteristics of tea bags include: color, aroma, and taste. The organoleptic test uses a numerical scale to assess the level of panelists' acceptance of the product. The method used is the preference test (*Hedonic test*) using 20 panelists. The panelists were asked to taste and rate the tea bag products according to the scoring sheet.

Antioxidant Activity Test
DPPH method principle

The ability of the test sample to immerse the DPPH free radical oxidation process in methanol solution (so that the DPPH color changes from purple to yellow) with an IC_{50} value (concentration of the test sample capable of reducing free radicals by 50%) as a parameter to determine the antioxidant activity of the sample (Molyneux, 2016).

Preparation of DPPH mother liquor

The DPPH solution was prepared by weighing 50 mg of DPPH then put in a 100 ml volumetric flask and made up to the mark with methanol.

Measurement of the maximum absorption wavelength of DPPH

A total of 2 ml of the DPPH solution was put into a volumetric flask and then filled with methanol until the marking line. Determine the maximum wavelength, measure the absorbance at a wavelength of 400-800 nm. The maximum absorbance was obtained as the DPPH wavelength and repeated 3 times.

DPPH *operating time* measurement

A total of 2 ml of the DPPH solution was put into a volumetric flask and then filled with methanol until the marking line. The absorbance was measured at the maximum wavelength and repeated 3 times.

RESULTS AND DISCUSSION

Tea Bag Preparation Formulation

Simplicia powder kecibeling leaves (*Strobilantes crispa* (L.) Blume), salam leaves (*Syzygium polyanthum* (Wight.) Walp.) and meniran herb (*Phyllanthus niruri* L.) formulated into 3 formulations (Table 1). Tea bag formulations were made with various concentrations, each of which was put in a tea bag (Figure 3).

Table 1. Simple combination formulation

No	Formula	Simplicia Combination Comparison			Tea Bag Weight (grams)
		Kecibeling leaves	Salam leaves	Meniran herb	
1	I	0.4	0.2	0.05	0.65
2	II	0.5	0.3	0.15	0.95
3	III	0.6	0.4	0.25	1.25

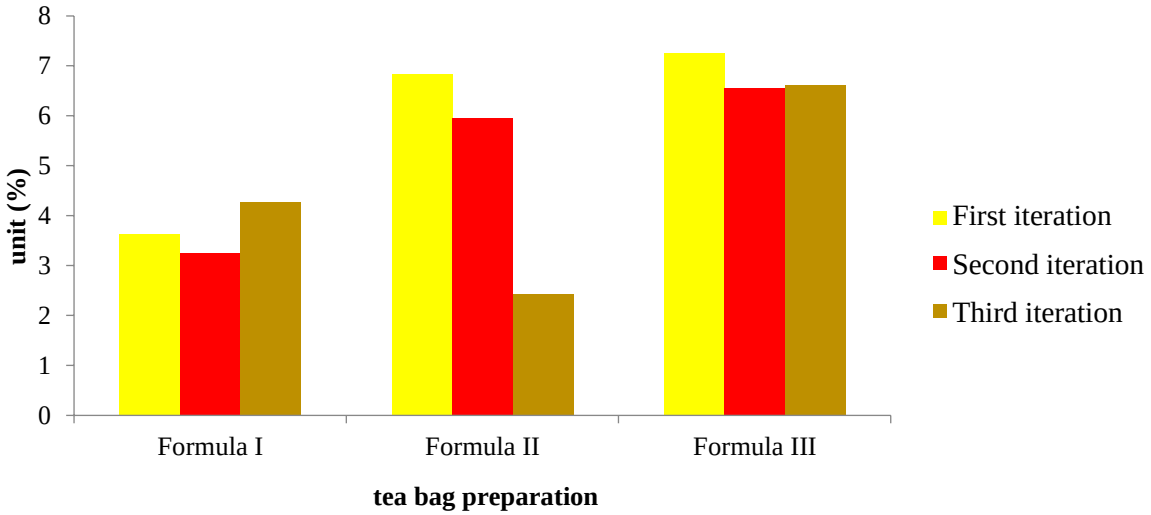
Formula I (0.65 g) Formula II (0.95 g) Formula III (1.25 g)

Figure 3. Preparation of tea bags

The formulations in (Table 1) were put in tea bags (Figure 3). Then the water content test, pH test preparation, organoleptic test (*Hedonic test*), and testing of antioxidant activity using the DPPH method.

Water content test results

Water content testing carried outin various concentrations is to meet the requirements (Depkes RI, 2000) namely: no more than 10%. Observation of water content aims to provide a minimum limit or range of the amount of water content in simplicia (Depkes RI, 2000). The results of this water content test give the average value for each formula, respectively, which is 3.72%; 5.07% and 6.81% (Graph 1).



Graph 1. Water content test results

pH test results

The pH of the preparation was determined using a pH meter. Measurement of the pH of the preparation was carried out on all teabag formulations. From the experiments conducted, the data obtained are in (Table 2).

Table 2. Data on the results of testing the pH of tea bag preparations

No	Formula	pH			Average
		1	2	3	
1	I	6.5	6.8	7.0	6.7
2	II	7.0	7.0	7.0	7.0
3	III	7.0	7.0	7.1	7.03

Information : 1: First iteration, 2: Second iteration, 3: Third iteration.

On (Table 2) shows that the preparation of tea bags from kecibeling leaves simplicia, salam leaves and meniran herb obtained pH results in the range of 6.7-7.0. This shows that the tea bag preparations meet the requirements of 4.0-7.0 (SNI 6986-11, 2019). The pH measurement aims to determine whether the pH of the formulated tea bag preparation is not too acidic or alkaline for consumption.

Organoleptic test results

On organoleptic testing the method used is the preference test (*Hedonic test*) using as many as 20 panelists or volunteers. From the 20 panelists who chose the preferred tea bag preparations based on color, aroma and taste, namely formula II tea bag preparations (Table 3).

Table 3. Preferred value data for tea bags

No.	Formula	Organoleptic Test			Information
		Color	Scent	Flavor	
1	I	3	3	3	Neutral
2	II	3	4	4	Like
3	III	3	4	2	Neutral

Description of Preferred Value: 1: Dislike very much, 2: Dislike, 3: Neutral, 4: Like, 5: Really like

The test results of the antioxidant activity of tea preparations dip

Testing the antioxidant activity of teabag preparations was carried out by UV-Vis spectrophotometry using the DPPH method (*2,2-diphenyl-1-picrylhydrazyl*). This method is the most simple, easy, fast method and only requires a small sample to evaluate the antioxidant activity of natural compounds.

The results of the measurement of the maximum absorption wavelength of DPPH

Testing of antioxidant activity begins with measuring the maximum wavelength of the DPPH solution in methanol using UV-Vis spectrophotometry in the wavelength range of 400-800 nm. The results of measurements carried out for 3 repetitions showed the maximum absorption of DPPH at a wavelength of 514 nm with an absorbance value of 0.530.

***Operating time* measurement results**

The results of the *operating time* measurement were carried out on the DPPH solution and formula II + DPPH each was repeated 3 times. At 8-12 minutes the DPPH solution showed a stable absorbance, and at 17-22 minutes the formula II + DPPH showed a stable absorbance, where the sample reacted perfectly with the DPPH solution.

The results of the analysis of the percentage of antioxidant activity

The antioxidant activity of formula II tea bag preparations can be seen in fig (Table 4).

Table 4. Antioxidant activity of formula II tea bag preparations

No	Sample Concentration (µg/mL)	Absorbance			Antioxidant Activity (%)		
		1	2	3	1	2	3
1	0		0.535			-	
2	95	0.280	0.285	0.247	47.66%	46.73%	53.83%
3	190	0.133	0.117	0.122	75.14%	78.13%	77.19%
4	380	0.083	0.095	0.095	84.48%	82.24%	82.24%
5	570	0.101	0.090	0.093	81.12%	83.17%	82.61%
6	760	0.110	0.089	0.083	79.43%	83.36%	84.48%
7	950	0.112	0.088	0.081	79.06%	83.55%	84.85%

Information: 1: First iteration**,** 2: Second iteration**,** 3: Third iteration.

On (Table 4) showed a decrease in absorbance at concentrations of 95 and 190 g/mL and a very significant decrease at a concentration of 380 g/mL, then at concentrations of 570, 760 and 950 g/mL an increase. This shows a non-positive relationship between concentration and absorbance levels. In (Table 4) it can also be seen that there was an increase in

antioxidant activity with a decrease in absorbance.

IC_{50} value analysis

The IC_{50} values for simplicia tea bags of kecibeling leaves (*Strobilantes crispa* (L.) Blume), salam leaves (*Syzygium polyanthum* (Wight.) Walp.) and meniran herb (*Phyllanthus niruri* L.) in formula II can be seen in (Table 5). The regression equation was obtained from the sample concentration data and % antioxidant activity.

Table 5. IC_{50} value of formula II tea bag preparations

No	Repetition	Regression Equation	IC50 (µg/mL)
1	I	Y = 0.05900x + 39.0030	186.39
2	II	Y = 0.06396x + 38.4025	181.32
3	III	Y = 0.06249x + 40.1653	157.37

In (Table 5) shows that the IC_{50} value of tea bag preparations in repetition I was 186.39 g/mL, repetition II was 181.32 g/mL and repetition III was 157.37 g/mL. From the results of the IC_{50} value obtained, the tea bag preparation is included in the "Medium" category as an antioxidant. The lower the IC_{50}, the higher the antioxidant activity (Figure 4).

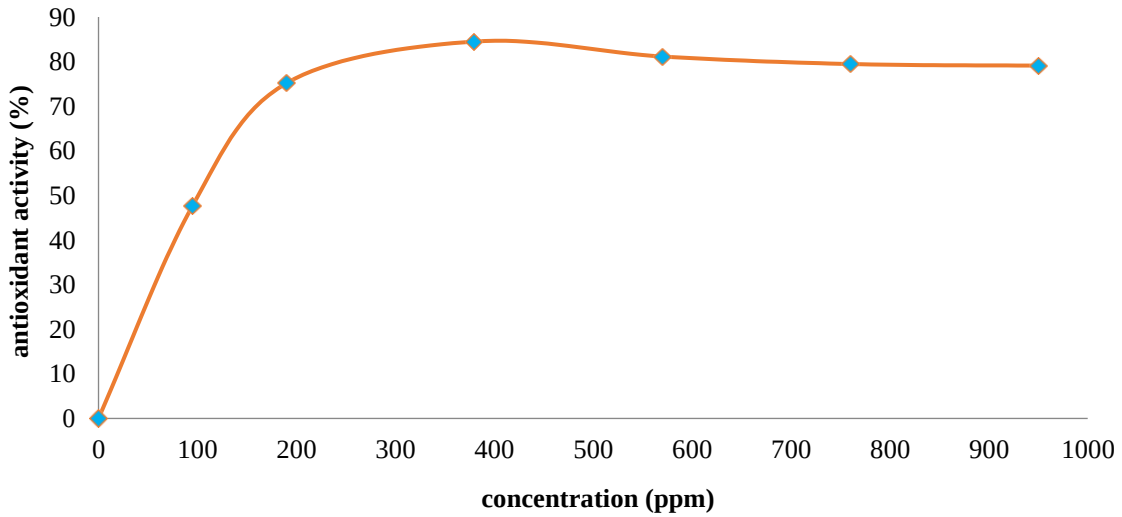


Figure 4. Concentration curve of formula II tea bag preparation with antioxidant activity

Table 6. Relationship of IC50 Value to Antioxidant Activity

No.	IC_{50}	Aktivitas Antioksidan
1.	<50 µg/ml	Sangan Kuat
2.	50 µg/ml	Kuat
3.	**101µg/Ml-250 µg/ml**	**Sedang**
4.	250 µg/Ml-500 µg/ml	Lemah
5.	>500 µg/ml	Tidak Aktif

CONCLUSION

The results obtained from testing the antioxidant activity of the formula for the combination of kecibeling leaf simplicia, bay leaf and meniran herb using a UV-Vis spectrophotometer at a wavelength of 515 nm have antioxidant activity with an IC50 value of 186.39 g/mL with the category "Medium" .

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