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Original Research

Antioxidant Activity Test on Sungkai Leaf *(Peronema canescens Jack)* Steeping Drink with the Addition of Honey

Uji Aktivitas Antioksidan pada Minuman Seduhan Daun Sungkai (Peronema canescens Jack) dengan Penambahan Madu

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Abstract: *Herbal Medicine in Indonesia have about 1,260 species in total. Mostly, many of the Indonesian from South Sumatra, use the sungkai plant (Peronema canescens Jack) to increase the body's immunity by making steeping drinks. Sungkai plants have secondary compounds such as alkaloids, flavonoids, and tannins which has function as antioxidants. The addition of honey as an alternative sweetener can be used to enhance the taste and also as an additional antioxidant.* ***Objectives:*** *This study aims to determine the antioxidant activity of sungkai leaf steeping drink with the addition of honey.* ***Methods:*** *This study was an experimental design and conducted in September-October 2021 at the Alma Ata University Food Laboratory and the Chemix Pratama Laboratory. Antioxidant activity analysis used the Dephenyl Picrylhydazil (DPPH) test on sungkai leaf steeping with the addition of honey. Fully randomized design and quantitative descriptive analysis with one-way ANOVA followed by Duncan's test.* ***Results:*** *Sungkai leaf steeping drink was tested for antioxidant activity to obtain an IC 50 value. In the control group (F0) without the addition of honey yield an IC50 value of 10,626.62 ppm, in the F1 treatment group with 1 g of Sungkai leaves and 8 ml of honey yield an IC50 value of 8,397.31 ppm, in the F2 treatment group with 1g of sungkai leaves and 10 ml of honey yield an IC50 value of 7,795.69 ppm, in the F3 treatment group with 2 g of sungkai leaves and 8 ml of honey yield an IC50 value of 8,189.88 ppm and in F4 treatment group 2 g of sungkai leaves and 10 ml honey yield an IC50 value is 7390.23 ppm. The F4 treatment group had the highest antioxidant activity with an IC50 value of 7,390.23 ppm.* ***Conclusion:*** *Steeping Sungkai leaf drink has antioxidant activity, however in very weak status. Further research needs to be done by modifying additional component to increase antioxidant activity in sungkai leaf steeping drinks.*

Keywords: antioxidant, steeping, sungkai leaf, honey

1. INTRODUCTION

Indonesia is a country of the third largest tropical forest in the world after Brazil and Zaire with abundant biological diversity. Biological diversity is the basis for various treatments and pharmaceutical industry findings in the future. The number of effective medicinal plants in Indonesia is estimated to be around 1,260 types of plants and herbs. One of the medicinal plants commonly used by the people of Indonesia is the sungkai plant (Peronema canescens Jack) (1). This plant is a typical Indonesian plant in southern Sumatra and Kalimantan (11). Empirically, sungkai leaves are used by some people to relieve toothache pain and reduce fever. Furthermore, Sungkai leaves are also used to cure malaria and have the potential to increase body immunity and increase the number of leukocytes (16).

The leaves of the Sungkai plant contain secondary metabolites such as alkaloids, flavonoids and tannins. The existence of these various compounds causes the presence of antibacterial activity in Sungkai leaves (16). Metabolite compounds that have antibacterial activity also generally function as antioxidants (2). Antioxidants are chemical compounds that in certain amounts can limit or slow down damage caused by the oxidation process, and are needed by the body to protect the body from free radicals.

The addition of honey as an alternative sweetener to Sungkai leaf steeping is expected to increase antioxidant activity in Sungkai leaf steeping, because one of the mineral contents in honey is manganese (Mn) which functions as an antioxidant and also the addition of honey to neutralize the bitter taste in Sungkai leaves (9). In this study, sungkai leaf steeping was used with the addition of honey, namely pure TJ honey with a concentration of 8 ml and 10 ml respectively (6). Therefore, the aims of the study was to determine the antioxidant activity of sungkai leaf steeping drink with the addition of honey

2. METHODS

Study Design

This study was experimental research and the design used was a single factor fully randomized design with five treatments and three repetitions. The treatment applied was to know is the antioxidant activity of leaf steeping with the addition of honey as F1, F2, F3 and F4 group. F0 as a control treatment for steeping, Sungkai leaves without the addition of honey. The research was conducted in September-October 2021 at the Food Laboratory of Alma Ata University and the Chemix Pratama Laboratory in Bantul, Yogyakarta. This research has been carried out and has received an ethical clearance letter (Ethical Clerence) from the Ethical Commission of Alma Ata University Yogyakarta (No: KE/AA/X/10632/EC/2021).

Sample of The Study

The ingredients used to sungkai leaf infusion with the addition of honey were taken from Tabepenanjung Village, Bengkulu City. 150 g pure of TJ honey purchased at a pharmacy and tap water which is cooked until it boils at 1000C. The material used in the analysis of antioxidant activity is a decoction of Sungkai leaves with the addition of honey. The chemicals used were 96% ethanol, filter paper, solution (2,2 Diphenyl-1 Picrihidrazil (DPPH)). The formulation of Sungkai leaves with the addition of honey can be seen in Table 1. The scheme for making Sungkai leaf infusion with the volume of the drink and the results were showed in Figure 1 and 2.

Table 1. Steeping Sungkai Leaves with Added Honey

Ingredients	F0	F1	F2	F3	F4
Simplisia	1 gr	1 gr	1 gr	2 gr	2 gr
Water	100 ml	100 ml	100 ml	100 ml	100 ml
Steeping Sungkai Leaf	100 ml	92 ml	90 ml	92 ml	90 ml
Honey	-	8 ml	10 ml	8 ml	10 ml
Volume Total	100 ml	100 ml	100 ml	100 ml	100 ml

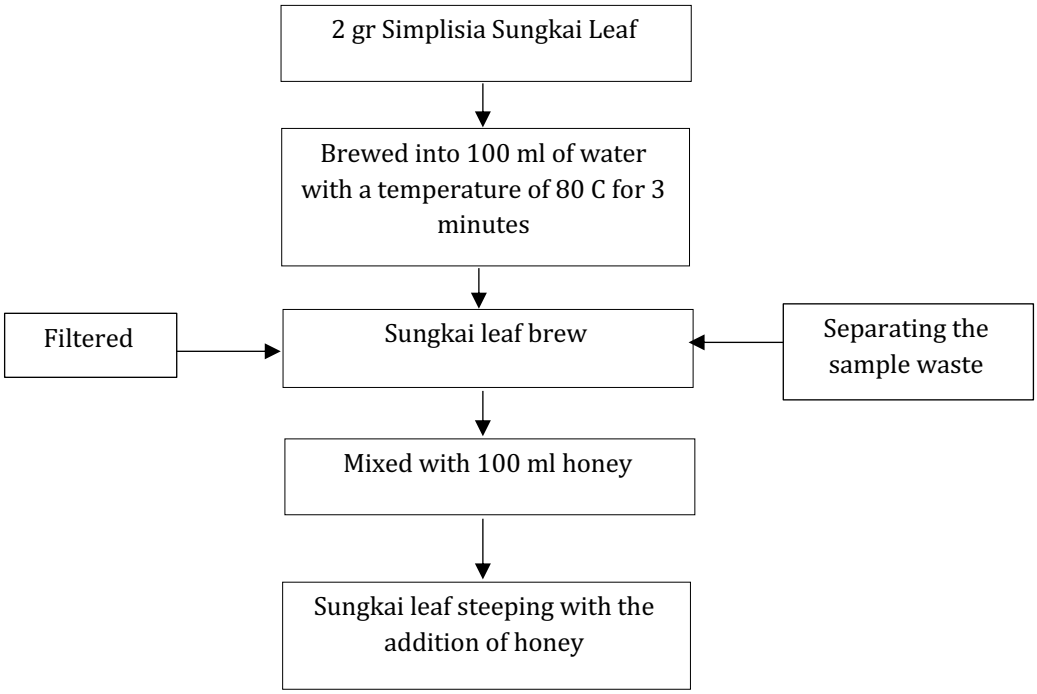

Figure 1. Scheme for Making a Sungkai Leaf Stew with the Addition of Honey

Antioxidant Activity Measurement

To measure the antioxidant activity in Sungkai leaf steeping with the addition of honey, as well as the percentage of antioxidant activity using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{blank absorbance}}{\text{Blank absorbance}} \times 100\ \%$$

The calculation for determining free radical scavenging activity is IC50 (Inhibitory Concentration), this value describes the concentration of the test compound that can trap free radicals by 50%. The IC50 value is calculated using the linear regression equation Y = ax + b. The sample concentration (ppm) is the abscissa (x-axis) and the % value of antioxidant activity is the ordinal (y-axis). to determine the IC50 value can be calculated using the formula:

$$\text{IC50} = \frac{50-b}{a}$$

Description =

a = Intercept (line intersection on the y axis)

b = Slope (slope)

Statistical Data Analysis

Data analysis in this study was descriptive quantitative to measure the antioxidant activity of sungkai leaf steeping with the addition of honey. The data that has been collected is then processed using Microsoft Excel and analyzed with SPSS using the One-Way ANOVA test to compare all treatments given to the sample.

3. RESULTS

Sungkai Leaf Morphology Test *(Paronema Canescens Jack)*

Based on the results of the determination of the sample used in this study, the sungkai leaves of *Peronema Canescens Jack* were taken in Tabepenanjung Village, Bengkulu City. These plants are identified in advance with the aim of ensuring the correctness of the plants used and preventing errors in the use of plants in research. The identification results of plant determination stated that the sample used in this study was *Peronema canescens Jack* which belongs to the *Lamiaceae family*.

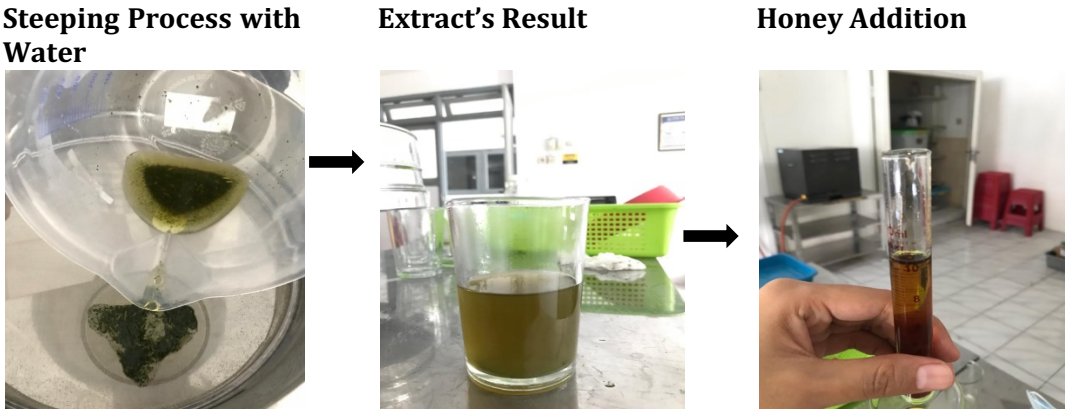

Figure 2. The Process of Making a Sungkai Leaf Stew with the Addition of Honey

Antioxidant Activity Test of Sungkai Leaf Stew with the Addition of Honey

The average value of IC50 antioxidant levels in Sungkai leaf steeping with the addition of honey can be seen in Table 2.

Table 2. Average Results of Antioxidant Activity of Sungkai Leaf Stew with Addition of Honey

Treatment	Antioxidant Activity IC50 (ppm)	P-Value
F0 (Controlled Formula)	10.626,20 ± 3.214,23[a]	
F1	8.397,31 ± 9.667,88[b]	
F2	7.795,69 ± 1.637,17[c]	< 0.001
F3	8.189,88± 1.065,13 [b]	
F4	7.390,23 ± 1.903,68 [d]	

a/b/c/d = Notation of different letters indicates a significant difference between each treatment (P <0.05) in the One-Way Anova test followed by Duncan's test. IC 50 = Inhibition Concentration.

Based on Table 1, the results of the One-Way Anova statistical test showed a p-value of <0.001, which means that there was a significant effect of steeping Sungkai leaves with the addition of honey on the antioxidant activity test (F0, F1, F2, F3, F4). The average antioxidant activity in steeping Sungkai leaves without adding honey to (F0) or the control formula was 10,626.20 ppm, in the treatment of adding 8 ml of honey and 1 g of Sungkai leaves (F1) was 8,397.31 ppm, in the treatment of adding 10 ml and 1 g of honey sungkai leaf (F2) was 7,795.69 ppm, in the treatment of adding 8 ml of honey and 2 g of sungkai leaf (F3) was 8,189.88 ppm, in the treatment of adding 10 ml of honey and 2 g of sungkai leaf (F4) was 7,390.23 ppm. Furthermore, it can be concluded that the addition of honey to the Sungkai leaf steeping shows a significant effect on antioxidant activity.

The results of the Duncan test showed that the treatment without adding honey to the control formula (F0) was significantly different from F1, F2, F3 and F4. The antioxidant activity test of F1 was not significantly different from F3, but F1 was significantly different from F0, F2 and F4, the results of the F2 test were significantly different from F0, F1, F3 and F4. The results of the antioxidant test for F3 were not significantly different from F1, but F3 was significantly different from F0, F2 and F4. The results of the F4 test were significantly different from F0 F1 F2 and F3. Furthermore, it can be concluded that the antioxidant activity of sungkai leaf steeping in the F0, F2 and F4 treatments was significantly different, while in F1 and F3 it was not significantly different.

4. DISCUSSION

Antioxidant Activity Test of Sungkai Leaf Stew

The antioxidant activity in this study was carried out using the DPPH free radical inhibitor method. This method was chosen because it is simple, easy and uses a small number of samples in a short time (3). The antioxidant activity in this study was carried out using the DPPH free radical inhibitor method. This method was chosen because it is simple, easy and uses a small number of samples in a short time (4). The results of antioxidant testing in this study showed that the activity of the IC50 antioxidants category was weak.

There is an effect of adding honey to Sungkai leaf extract on antioxidant activity. Antioxidants were expressed with IC50 for each sample differently in the F4 treatment with an average result of 7390.23 ppm in the weak category, in the F3 treatment with an average result of 8189.88 ppm in the weak category, while in the F2 treatment with an average result of 7795.69 ppm the weak category, the F1 treatment was 8397.31 ppm the weak category, while the F0 treatment was the control treatment with an average value of 10626.20 ppm the weak category.

The results of research conducted by Fadlillahturahmah (2021) The results show very active free radical inhibitory activity. the IC50 value of the methanol extract of Sungkai leaves was 9.389 ppm. Differences in IC50 values are due to differences in DPPH concentrations, sample solvents used and sample growing areas (7). Quantitative antioxidant activity of P. canescens leaves, where the Peronema canescens jack plant was extracted with methanol solvent and tested for its antioxidant activity using the DPPH method showed that the methanol extract of the P. canescens plant had an antioxidant activity of 49.5±1.21mg/mL (10).

The test results for the steeping of Sungkai leaves have a weak antioxidant activity value but have differences in each sample. This is because the process of extracting sungkai leaves only uses water, not through the process of ethanol extraction or maceration. This research is in line with research conducted by Anisa Fitria (2021) the ethanol (polar) extract of Sungkai leaves has an IC50 value of 44.933 ppm, which shows that Sungkai leaf extract has very strong antioxidant activity. This is different from the results obtained, possibly due to differences in the content of secondary metabolites in plants depending on the extraction method used. The content of secondary metabolites in plants is closely related to antioxidant compounds. Differences in the extraction technique of a simplisia will greatly affect the content and quality of the secondary metabolites produced (8).

The Addition of Honey to a Stew of Sungkai Leaves

The addition of honey can affect the ability to ward off DPPH free radicals and the results of statistical tests show that the difference in the concentration of honey added to the Sungkai leaf extract drink gives a significant difference in each sample treatment. The ability to ward off free radicals is influenced by the presence of total phenols and flavonoids contained in the sample. Other compounds such as enzymes and organic acids in honey, as well as the synergistic interaction of compounds in honey and Sungkai leaf extract can also support the ability of antioxidant activity (14).

Honey contains phenolic compounds because, during its production, bees mix their bodily fluids with floral nectar or plant secretions consisting of water, sugars, proteins and phenolic compounds. These compounds have shown significant biological activity in the treatment of various diseases. The interaction of phenolic compounds with the gut microbiota as well as beneficial microorganisms (probiotics) is an emerging factor in achieving the health-promoting effects induced by these compounds. Foods, such as honey, which contain phenolic compounds, are prebiotics and have a good interaction with probiotics, present a very attractive added value in various industries, such as the food industry because of their acceptance and health benefits (5).

In addition to phenolic compounds, honey contains a number of minerals such as magnesium, potassium, potassium, sodium, chlorine, sulfur, iron, phosphate and vitamins, such as vitamin E, vitamins B1, B2, B6 and vitamin C. Vitamin C is the main antioxidant compound in plasma against free radical attack (ROS) and also plays a role in cells. In addition, honey also contains many benefits and properties contained in it (15).

Honey has health benefits, one of the criteria for good quality honey is the presence of enzyme activity in honey. The enzymes present in honey are invertase, diastase, peroxidase and protease enzymes. The invertase enzyme functions to break down sucrose into glucose and fructose. While the diastase enzyme is an enzyme that functions to convert starch into dextrin and maltose. Peroxidase enzymes play a role in catalyzing hydrogen and also as hydrogen ion acceptors (13).

Honey antioxidant activity test resulted in an absorbance value which always decreased with increasing sample concentration. Based on the results of the honey antioxidant activity test, it showed that the higher the sample concentration, the higher the DPPH violet color decay which resulted in a decrease in the absorbance value of the sample at each increase in concentration. Antioxidant activity was measured as a result of a decrease in the absorption of DPPH solutions due to the addition of samples (12).

Factors that affect this antioxidant activity also include the different composition of honey which depends on the floral source used for collecting nectar by honey bees, seasonal and climatic factors, and processing (12). Based on the results of testing the antioxidant activity obtained in this study, it is known that the steeping of sungkai leaves with the addition of honey has a very weak level of antioxidant activity. Even so, the antioxidant activity of sungkai leaf steeping with the addition of honey still has significant antioxidant activity so that it has a function as an antioxidant. In the process of making Sungkai leaf extract, it is not maximally using the water solvent method so that the results of antioxidant activity are very small.

5. CONCLUSION

Sungkai leaf steeping without the addition of honey or in the control formula F0 had a very weak antioxidant value with an IC50 value of 10,626.20 ppm, while Sungkai leaf steeping with the addition of 10 ml honey and 2 g concentration of Sungkai leaves increased antioxidant activity with an IC50 7,390.23 ppm that is in the F4 treatment group, but the antioxidant content in weak category with an IC50 > 200 ppm. Further research needs to be done by modifying it to increase antioxidant activity to be able to develop this compound into medicine or supplement ingredients.

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