

Analysis of the Effect of Maceration and Soxhletation Extraction Methods on the Antioxidant Activity of Sungkai Leaf Ethanol Extract (*Peronema canescens* Jack)

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Abstract

Sungkai leaves (*Peronema canescens* Jack) are one of the medicinal plants that have antioxidant activity, in the form of phenolic compounds, flavonoids, and alkaloids. The differences in the characteristics of extraction methods can have different influences on the antioxidant activity contained in the extract. This study aims to determine the class of secondary metabolite compounds and antioxidant activity of ethanol extract of sungkai leaves extracted by maceration and soxhletation methods and to determine the effect of maceration and soxhletation extraction methods on the antioxidant activity of ethanol extract of sungkai leaves. Antioxidant activity testing was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method as a free radical and using a UV-Vis Double Beam spectrophotometry instrument. Statistical test using SPSS 26 with the One Way ANOVA analysis test. The results of the study obtained ethanol extract from sungkai leaves extracted by maceration and soxhletation methods positively contained alkaloids, flavonoids, phenols, tannins, saponins and terpenoids. Average score obtained from ethanol extract of sungkai leaves maceration method is 25.2187 µg/mL while the soxhletation method is 18.9279 µg/mL. The results of the statistical test were obtained with a sig value of 0.001.

Keywords: *Peronema canescens* Jack; Maceration; Soxhletation; Antioxidant

1. Introduction

The Sungkai plant (*Peronema canescens* Jack) is spread in several areas, especially in West Sumatra, Jambi, Bengkulu and Lampung. Empirically, sungkai plants have been widely used as a fever reducer, toothache medicine, malaria, worms and minor wound medicine (Ahmad & Ibrahim, 2015). Meanwhile, pharmacologically, sungkai plants can be used as antihyperuricemia (Latief *et al.*, 2021). Sungkai plants have bioactive compounds in the form of phenolics, tannins, alkaloids, flavonoids, steroids, saponins, where these compounds have antioxidant activity (Pindan *et al.*, 2021).

Antioxidants are compounds that are able to prevent oxidation caused by free radicals. Free radicals have an influence on the health of the human body (Unsal *et al.*, 2021). The antioxidant activity of the ethyl acetate fraction of sungkai leaves (*Peronema canescens* Jack) by the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH) has very strong antioxidant activity with 44.933 µg/ml (Pindan *et al.*, 2021). Ethanol extract of young sungkai leaves with an IC₅₀ value of 50.838 µg/ml, old sungkai leaves 52.835 µg/ml, so it is included in the category of strong antioxidants (Okfrianti & Irnamera, 2021). Effect of heat extraction method on antioxidant activity in cashew leaf extract (*Anacardium occidentale* L.) using reflux and soxhletation methods of values 9.56 µg/ml and 9.08 µg/ml, very strong activity category (Warnjis *et al.*, 2020). Guava leaf testing using maceration and soxhletation extraction methods has very strong antioxidant activity with 47.80 µg/ml and 37.67 µg/ml (Nurhasnawati *et al.*, 2017).

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2. Methods

The tools used include: The tool used in the research is the UV-Vis Spectrophotometer (Shimadzu UV-1800), rotary evaporator (Heidolph), oven (Mettler), analytical scales (Ohaus). Sungkai leaves (*Peronema canescens* Jack.), DPPH (1,1-diphenyl-2-picrylhydrazyl) (Himedia), ethanol (C₂H₅OH) 70% (PT. Novalindo®), aquadest (H₂O) (PT. Novalindo®)

2.1. Procedure

The Sungkai leaves used are derived from Kelurahan Kurao Pagang, Kelurahan Parak Rumbio, Kecamatan Nanggalo, Kota Padang Sumatera Barat as much as 2 Kg. Sungkai Plant Identified in Herbarium Universitas Andalas (ANDA) Jurusan Biologi FMIPA Universitas Andalas, Padang, Sumatera Barat. The dry simplicia processing process goes through the stages of raw material collection, wet sorting, washing, cracking, drying, dry sorting, and storage (Indonesia, 1985).

2.2. Manufacture of Sungkai Leaf Ethanol Extract Maceration and Soxhletation Method

Sungkai leaf ethanol extract is made by maceration and soxhletation methods. Maceration is carried out Weighing 200 g of dried powder of simplicia sungkai leaves macerated with 2000 mL of 70% ethanol (1:10) in a dark bottle. Soak for the first 6 hours while stirring occasionally, then let it sit for 18 hours. Repeat the refining process three times with the same type and amount of solvent. Collect all the mafibers, then steam using a rotary evaporator at a temperature of about 50°C until a thick extract is obtained (Indonesia, 2008).

As for the socle method, a scretization device is installed, then a sample of 200 grams is wrapped in filter paper and tied with thread, put into a soclet device, input 70% ethanol solvent as much as 2000 mL. Socle until the cycle droplets are colorless or approximately for 13 hours and the cycle is calculated. The liquid extract obtained is then concentrated with using a rotary evaporator at 50°C (Wijaya *et al.*, 2022).

2.3. Manufacture of DPPH solution 30 µg/mL

Carefully weighed approximately 10 mg of DPPH (BM 394.33). It is then dissolved with methanol p.a up to 100 mL, then placed in a measuring flask coated with aluminum foil. Suffice the solvent to the limit mark then shake until homogeneous and obtain a DPPH solution with a concentration of 100 µg/mL. Then diluted by being squeezed by 15 mL of DPPH solution with a concentration of 100 µg/mL, put it in a 50 mL measuring flask, sufficiency of the solvent until the boundary mark and then beat until homogeneous and obtained a DPPH solution with a concentration of 30 µg/mL (Molyneux, 2004).

2.4. Manufacture of Maximum Clump Length Optimization Blank Solution DPPH

Pinch 3.8 mL DPPH 30 µg/mL into the vial, add 0.2 methanol p.a, then homogenize and cover the vial with aluminum foil. Then it was incubated in a dark room for 30 minutes. Determine the maximum absorption wavelength of DPPH using a UV Vis spectrophotometer at a wavelength of 400-800 nm (Andayani *et al.*, 2008).

2.5. Antioxidant Activity of Sungkai Leaf Ethanol Extract By Maceration And Soxhletation Method

Weighing 100 mg of sungkai leaf extract, then dissolved with methanol p.a in a measuring flask ad 100 mL, a concentration of 1000 µg/mL was obtained. Then dilute by pipetting 5 mL of a solution of 4, 8, 12, 16 and 20 µg/mL is made by pipetting 0.4, 0.8, 1.2, 1.6 and 2 mL from a 100 µg/mL solution, each of which is put in a 10 mL measuring flask and methanol p.a is added to the limit mark. For the determination of antioxidant activity, each concentration was pipped as much as 0.2 mL of sample solution and put it into a vial, then added 3.8 mL of DPPH solution 30 µg/mL. The mixture is homogenized and left for a long time 30 minutes in a dark place, absorption is measured with a UV-Vis spectrophotometer at the maximum absorption wavelength DPPH. The antioxidant activity of the sample is determined by the magnitude of the DPPH radical absorption barrier through percentage calculation DPPH absorption inhibition (Andayani *et al.*, 2008)

3. Results and Discussion

The manufacture of the extract is carried out by maceration and soxhletation methods. The selection of the maceration method because the procedures and equipment used are simple while the selection of extraction methods using scleration is one of the best methods used in separating bioactive compounds from nature and has advantages such as less solvents used, shorter extraction time and samples that are extracted perfectly because they are done repeatedly (Molyneux, 2004). The extraction is carried out using ethanol solvents that are polar because they can dissolve

antioxidant components which are secondary metabolites in sungkai leaves. The maserrate obtained is then concentrated using a rotary evaporator until a thick extract is obtained.

The antioxidant activity of a compound is indicated by the absorption barrier of DPPH having strong absorbance at wavelengths of 515-517 nm with a dark violet color (Molyneux, 2004). In is 515.00 nm with an absorbent of 0.558 can be seen in Figure 1. Hydrogen atom donation to DPPH will change the radical form of DPPH to non-radical which can be visually observable through its color change, if donated hydrogen atoms DPPH will change to non-radical which is characterized by fading from purple to lighter to yellow (Molyneux, 2004).

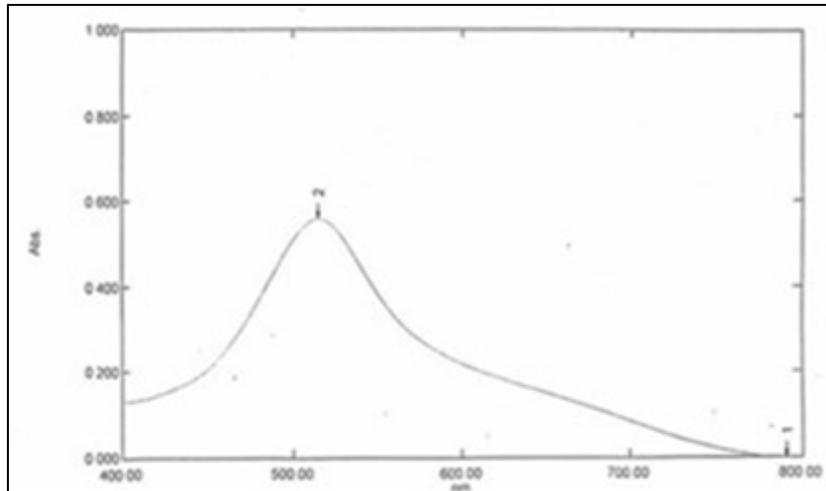


Figure 1 Maximum Wavelength 2,2-diphenyl-1-picrihydrazil

In testing the antioxidant activity of ethanol extract of sungkai leaves by the extraction method of maceration and soxhletation was carried out with three replications. The test was carried out three times to minimize sample analysis errors and measurement of antioxidant activity. Nilai IC50 obtained from ethanol extract of sungkai leaves by maceration extraction method of 24.5896 µg/mL, 24.3522 µg/mL, 26.7144 µg/mL can be seen in table 2. Based on (Molyneux, 2004). The results obtained show antioxidant activity with a very strong category due to the value $IC_{50} < 50 \mu\text{g}/\text{L}$. And Values IC_{50} obtained from ethanol extract of sungkai leaves by the extraction method of scleration of 18.9958 µg/mL, 18.5304 µg/mL, 19.2576 µg/mL obtained 18.9958 µg/mL can be seen in table 2. Based on (Molyneux, 2004). the results obtained showed antioxidant activity with a very strong category due to the value $IC_{50} < 50 \mu\text{g}/\text{mL}$.

Table 1 Antioxidant Activity of Ethanol Extract Of Sungkai Leaf Maceration Method

Replikasi	Konsentrasi	Absorban	%Inhibisi	IC50 (µg/mL)	Rata-Rata IC50 (µg/mL)
1	4	0,536	3,9426	24,5896	
	8	0,490	12,1863		
	12	0,437	21,6845		
	16	0,384	31,1827		
	20	0,338	39,4265		
2	4	0,532	4,6594	24,3522	25,2187
	8	0,486	12,9032		
	12	0,433	22,4014		
	16	0,382	31,5412		
	20	0,334	40,1433		
3	4	0,552	1,0752	26,7144	
	8	0,505	9,4982		
	12	0,454	18,6379		
	16	0,400	28,3154		
	20	0,365	34,5878		

Table 2 Antioxidant Activity of Ethanol Extract Of Sungkai Leaf Soxhletation Method

Replikasi	Konsentrasi	Absorban	%Inhibisi	IC50 (µg/mL)	Rata-Rata IC50 (µg/mL)
1	4	0,548	1,7921	18,9958	
	8	0,479	14,1577		
	12	0,408	26,8817		
	16	0,338	39,4265		
	20	0,256	54,1218		
2	4	0,541	3,0465	18,5304	18,9279
	8	0,471	15,5913		
	12	0,394	29,3906		
	16	0,331	40,6810		
	20	0,249	55,3763		
3	4	0,528	5,3763	19,2576	
	8	0,465	16,6666		
	12	0,401	28,1362		
	16	0,330	40,8602		
	20	0,267	52,1505		

The results of the antioxidant activity test showed that the ethanol extract of the mullet leaves from the mullet from the mullet provided a higher value than the ethanol extract of the mullet leaves from maceration. Extracts from soxhletation methods have very strong antioxidant activity compared to maceration results, this can happen due to the influence of extraction temperature, where by socling the extraction temperature can be adjusted so as not to damage the required antioxidant components. With the addition of extraction temperature, the required antioxidant components can be extracted Perfect so that the more components are dissolved, the greater the antioxidant activity (Wijaya *et al.*, 2022). The temperature in the soxhletation process affects the phenolic compounds that are drawn. The higher the temperature ($\leq 100^{\circ}\text{C}$) of extraction, the solubility of phenolic compounds increases (Mokoginta *et al.*, 2013). At 70°C flavonoids can still be extracted well, but at higher temperatures, such as 100°C , the flavonoid levels obtained tend to be lower. Although both methods show very strong antioxidant activity, it can be concluded that the difference in extraction methods affects the antioxidant activity produced. Furthermore, the Anova test was carried out using the Sum of squares test to find out whether there is an influence between the extraction method of maceration and soxhletation methods on antioxidant activity. The p value obtained from ethanol extract of sungkai leaves by the maceration and soxhletation method is 0.001 so it can be concluded that statistically the extraction method has an effect on the activity of antioxidants can be seen in table 3.

Table 3 ANOVA Test

ANOVA					
IC50	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59.361	1	59.361	64.964	.001
Within Groups	3.655	4	.914		
Total	63.016	5			
Nilai sig < 0,05					

The data presented in the research results is not in the form of raw data, but data that has been processed. The results are presented in the form of a table or image that is accompanied by an explanation. The explanation does not restate e

4. Conclusion

The secondary metabolite compounds contained in ethanol extract of sungkai leaf (*Peronema canescens* Jack) extracted by maceration and soxhletation methods are alkaloids, flavonoids, phenols, tannins, and saponins.

The antioxidant activity of ethanol extract of sungkai leaves (*Peronema canescens* Jack) extracted by maceration and soxhletation methods belongs to a very strong category with a value IC₅₀ best antioxidant extracted with soxhletation method.

There is an influence of the extraction methods of maceration and soxhletation on the activity of Antioxidant Extract of Ethanol Extract of Sungkai Leaves with a P value of < 0.05.

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