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Phenolic Content and Antioxidant Activity Water and Ethanol Extracts of Sungkai Leaves (*Peronema canescens* Jack)

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Abstract. *Sungkai (Peronema canescens Jack) is a plant that has phenolic content as an antioxidant that can enhance the immune system (immunomodulator). This study aimed to determine the phenolic content and antioxidant activity of Sungkai leaves (Peronema canescens Jack). The method of antioxidant with invitro using of the DPPH where the ability of a compound to inhibit DPPH radicals is calculated from the absorbance value using UV-Vis spectroscopy. And Determination of total phenolic content was carried out using UV VIS spectroscopy at a wavelength of 745 nm with the help of Folin Ciocalteu reagentand a nd gallic acid standard curve . The test results showed that the water and ethanol extracts with a concentration of 0.1% had a phenolic content of 0.002% and 0.007%, their antioxidant activity (IC50) with a concentration of 0.02; 0.04; 0.06; 0.08; and 0.1% in the water and ethanol extracts of Sungkai leaves have values of 0.025% and 0.03% respectively. Therefore the best antioxidant activity (IC50) was extracts ethanol of sungkai leaves with phenolic content of 0.007% and IC50 of 0.03%.*

Keywords: *covid-19, IC50, immunomodulator, phenolic,*

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INTRODUCTION

Humans and plants have a close relationship in life. As explained in QS. Abasa (80): 27-32, that the benefits humans can get from plants are numerous. However, not all plants created by Allah SWT on this earth have been successfully explored for the structure of compounds and their uses, especially in the field of medicine. In accordance with his words in the Qur'an, Allah SWT says that the creation of plants on earth is a blessing and favor given to all creatures. One of the plants that have been used by the people of South Sumatra (Palembang City) in preventing COVID-19 is the sungkai plant.(Kementrian Kesehatan RI, 2020).

The sungkai (*Peronema canescens* Jack) is a plant originating from Kalimantan. Sungkai is also spread in South Sumatra, West Sumatra, Bengkulu, Jambi and West Java (Fransisca et al, 2020). The sungkai plant is a wild plant with high economic value as it is made for sungkai leaves tea and drugs, so people cultivate it and use it as traditional medicine. Several studies have reported the

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content of secondary metabolites of Sungkai leaves such as steroids, alkaloids, terpenoids, flavonoids, tannins, saponins, and phenolics (Latief et al., 2021).

The phenolic compounds can potentially counteract free radicals and have antioxidant activity (Dillasamola et al., 2021, Maretha, 2022). Free radicals are compounds that have unpaired electrons making the compound very reactive and impacting the human body (Pindan et al., 2021; Sharma & Bhat, 2009). The effects produced by free radicals can be stopped with antioxidants, one of which is antioxidants derived from sungkai leaves. Antioxidants are compounds that can neutralize free radicals and prevent damage to cells, as well as boost the immune system as immunomodulators (Kurniasih et al. 2015). Immunomodulators are substances that can induce, strengthen, and inhibit components of the immune system (Dillasamola et al., 2021)

Sungkai leaves which contain secondary metabolites such as phenolic groups are thought to be able to play a good role as antioxidants which can enhance the immune system as immunomodulators (Yuniarti et al., 2020). However, based on a literature review, phenolic compounds tend to be attracted to polar solvents, due to the presence of hydroxyl groups, such as water and ethanol, but until now there has been no research using aqueous extracts of sungkai leaves (Chen et al., 2020). Few data only report the use of sungkai leaves extract with methanol, ethanol, and n-hexane solvents. This organic material is thought to have toxic properties to be used directly by people as herbal traditional medicine. Based on this, further research is needed to see the content of compounds that act as immunomodulators in sungkai leaves.

MATERIALS AND METHODS

This research was conducted at the Integrated Laboratory, State Islamic University Raden Fatah Palembang in February-May 2022.

Extraction

Sungkai leaves Simplicia of 20 g was put into a container and soaked in 200 ml of 96% ethanol, closed to protect from light and left for 24 hours, while stirring occasionally, then filtered to obtain a filtrate. The water solvent extraction method used in this research was maceration. Simplicia of 20 g was put in a container, then added 200 ml of distilled water, covered to protect it from light and leave it for 24 hours, while stirring occasionally, then filter it to obtain a filtrate. The filtrate obtained was then used to carry out phytochemical tests, determine phenolic content, and test antioxidant activity (Yani & Dirmansyah, 2021).

Phytochemical Test

The species of undergrowth observed under the stands were presented in tabular form containing local names, scientific names, and families, and a brief description of the benefits of the plants was provided. The species of trees that make up the stands were presented in tabular form containing local names, scientific names, and the commodities produced.

Determination of Phenolic Content

The ethanol extract and water of sungkai leaves with a concentration of 0.1% (v/v) were taken 1 ml eac, then 1 ml of Folin Ciocalteu The mixture was allowed to stand for 3 minutes and then mixed with 2 ml of Na2CO3 5%. Once homogeneous, let it stand again at room temperature for 60 minutes. Once homogeneous, let it stand again at room temperature for 60 minutes. All solutions were measured for their absorbance using a UV-Vis spectrophotometer at a length of 745 nm. After obtaining the absorbance value, the phenol

content was calculated using the linear regression formula obtained on the gallic acid standard curve (Tahir et al., 2017).

Antioxidant Activity Test

A solution of the ethanol and water extracts of each sungkai leaves was prepared at a concentration of 1% (v/v), and then dilution was carried out with various concentrations, namely a concentration of 0.02%; 0.04%; 0.06%; 0.08%, and 0.1%. Then the solution was added to 50 ppm DPPH solution with a volume ratio 1:1. The mixture was incubated for 30 minutes. The absorbance was measured using UV-Vis spectrophotometry at the maximum wavelength. Per cent Inhibition Concentration (IC50) can be calculated using the equation (Din et al., 2015):

$$\%\,Inhibisi = \frac{A\,B - A\,A}{A\,B} \times 100\%$$

where, AB = absorbance of DPPH solution (t = 0 min); AA = absorbance of tested extract solution (t = 30 min). The concentration of extract or standard which exhibited 50% radical scavenging (IC50) was deduced from the linear regression of concentration versus the percentage of inhibition (Mensor et al., 2001 ; Abimanyu, 2016).

RESULTS AND DISCUSSION

Phytochemical Test

The phytochemical test was conducted to determine the chemical compounds contained in the ethanol extract and aqueous extract of sungkai leaves. No one has studied the phytochemical test results of sungkai leaves water extract to what extent the phytochemical tests mostly use ethanol solvent. The following results of the phytochemical test are presented in Table 1.

Table 1. Phytochemical test results of sungkai leaves (*Peronema canescens* Jack)

Class	Test Results		Fransisca, et al, 2020	Latief et al, 2021
	Water	Etanol	Etanol	Etanol
Tannins	(+)	(+)	(+)	(+)
Flavonoids	(+)	(+)	(-)	(+)
Alkaloid	(+)	(+)	(+)	(+)
Saponins	(+)	(+)	(+)	(+)
Terpenoid	(-)	(-)	(-)	not
Steroid	(-)	(+)	(+)	(+)

Based on the research by (Latief et al., 2021), the same results were also obtained in the phytochemical test of the ethanol extract, which positively contained flavonoids, alkaloids, tannins, steroids and saponins. Meanwhile, the results from (Fransisca et al., 2020) showed that the ethanol extract of Sungkai leaves was negative for flavonoid.

Based on Table 1, the water extract of sungkai leaves has the same content as the ethanol extract except the negative steroid test. This is because ethanol and water are polar solvents. According to the polarization principle, a compound will dissolve in a solvent that has the same level of polarity (Sari & Yani, 2021). In the phytochemical test, flavonoids and tannins belong to the phenolic group of secondary metabolites. Based on a literature review on the results of isolating compounds found in sungkai leaves is 5,7-dihydroxy isoflavone, which is a group of secondary metabolites of a type of flavonoid that acts as an antioxidant

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(Dasrinal 2022).

Determination of Phenolic Content

Phenolic compounds are one of the most abundant secondary metabolites in plants. Phenolic compounds have conjugation bonds that can stabilize free radicals. Apart from that, this derivative compound has many hydroxyl groups (OH) which can donate electrons to free radicals (Fatahillah et al., 2022). The presence of phenolic compounds can be seen from the change in color of the test solution to blue. The blue color formed will be more concentrated in proportion to the phenolic ions formed, meaning that the greater the concentration of phenolic compounds, the more phenolic ions will reduce hetero-poly acids resulting intense blue color (Maigoda et al, 2022). The addition of sodium carbonate (Na2CO3) serves to create an alkaline environment because the folin ciocalteu reagent is not stable enough in an alkaline environment, and to reduce the foline by the hydroxyl groups of the phenolic in the sample.

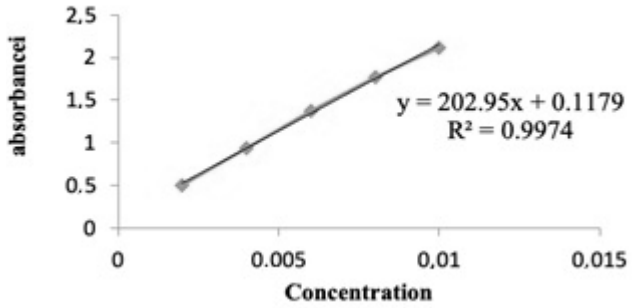

Figure 1. Gallic acid standard curve

Based on the regression equation that has been obtained in Figure 1, the equation used to determine the phenolic content was y= 202.95x+0.1179. with linearity r = 0.9974. The value of r indicates the value of linearity, namely the correlation between the concentrations of the resulting absorbance. The better the linearity value (r=1 or close to 1), the better the correlation. The absorbance of the sample obtained was then entered into the equation to obtain the value of the phenolic content in the sample.

Table 2. Phenolic content of water and ethanol extracts of sungkai leaves

Concentration	Ethanol Extract	Water Extract
0.1% (v/v)	0.007%	0.002%

From the linear regression formula (Table 2), the calculation results showed that the water extract of sungkai leaves at a concentration of 0.1% (v/v) has a phenolic content value of 0.002%, while the ethanol extract has 0.007%. The phenolic content of the ethanol extract was greater than that of the aqueous extract because although both solvents are generally polar, 96% ethanol is more effective in dissolving phenolic compounds in sungkai leaves than water. To confirm the phenolic content, analysis was carried out with an infrared (IR) spectrophotometer.

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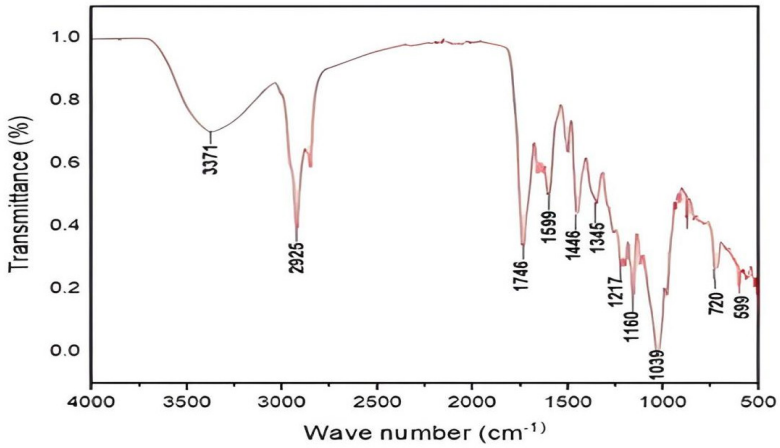

Figure 2. FTIR spectrum of sungkai leaves ethanol extract

According to research by Maigoda et al. (2022). signal of phenolic content, which has antioxidant properties, can be found in the range of 1680-900 cm-¹. The presence of phenolic compounds was based on the presence of C-O phenol groups at wave numbers 1160 and 1217 cm-¹, and was reinforced by the presence of aromatic C=C groups (1446 cm cm-1) and O-H groups (3371 cm-¹) (Figure 2).

Antioxidant Activity Test

Antioxidant activity testing can be done using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. This method uses free radicals in the form of DPPH. DPPH acts as a scavenger of free hydrogen radicals or captures electrons originating from a compound that can donate electrons (Sethi et al., 2020). Each molecule that can donate electrons or hydrogen will react and cause a decrease in the color intensity or DPPH absorbance. The DPPH color will change from purple to yellow. Table 3 shows the average absorbance and inhibition percentage of the aqueous and ethanol extracts of sungkai leaves:

Table 3. Absorbance value and percent inhibition

Concentration	Absorbance		% Inhibition	
	Water Extract	Ethanol Extract	Water Extract	Ethanol Extract
0.02%	0.444	0.438	48.07	48.77
0.04%	0.421	0.380	50.76	55.55
0.06%	0.379	0.309	55.67	63.85
0.08%	0.352	0.233	58.83	72.24
0.1%	0.333	0.148	61.05	82.69

From Table 3, the ethanol and water samples had a fairly good correlation with r values of 0.9952 and 0.9857, respectively, where the r value illustrates the linearity of concentration to percent inhibition. IC50 is the concentration to reduce 50% of free radi-

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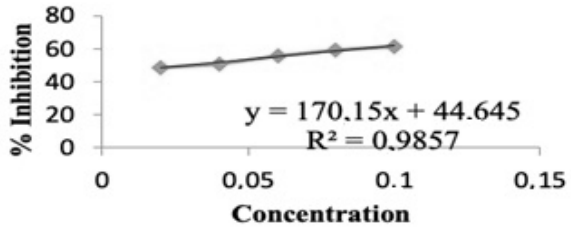

Figure 3. Relationship between concentration and percent inhibition of aqueous extract

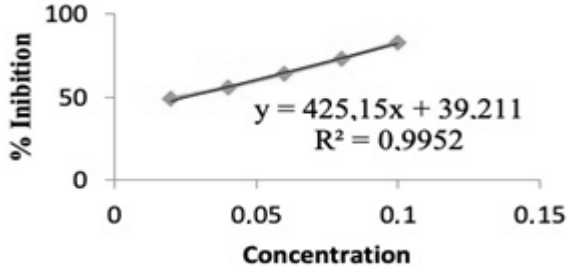

Figure 4. Relationship between concentration and percent inhibition of ethanol extract

Table 4. IC50 results of water and ethanol extracts of sungkai leaves

Value	Ethanol Extract	Water Extract
IC_{50}	0.025%	0.03%

In Table 4 above, the ethanol extract, in 0.0025 ml of sample with a volume of 10 ml, can inhibit 50% of the radicals, while the aqueous extract in 0.003 ml of the sample with a volume of 10 ml can inhibit 50% of the radicals. From Figure 3 and 4 that we can see the correlation between concentration and % inhibition from the extract. This is in line with the phenolic content, the higher the phenolic content, the greater the antioxidant activity. The greater the antioxidant activity, the smaller the IC50 value. It can also be seen that the IC50 values of the two extracts were not significantly different, this was allegedly because based on the phytochemical test results the water and ethanol extracts were positive for containing the same compounds, namely tannins, flavonoids, alkaloids, and saponins. Metabolite compounds such as flavonoids and tannins have conjugated double bonds and hydroxyl (-OH) groups which can provide electrons to stabilize free radicals (Saleem, Saleem, & Akhtar, 2020), while alkaloids have nitrogen (N) atoms that are rich in electrons so they can act as electron donors to radical atoms so they can act as antioxidant compounds (Fitri Yani & Dirmansyah, 2021).

CONCLUSION

The content of phenolic compounds at a concentration of 0.1% in the water extract was 0.002%, and in the ethanol extract was 0.007%. The IC50 value of the ethanol extract and the water extract did not differ much, but the IC50 value of the ethanol extract was smaller indicating better inhibition than the water extract.

AUTHOR CONTRIBUTION

The author, L. S. and D. E. M. designedresearch models, prepared tools and materials, oversaw the research process, collected and analyzed data, compiled and finalized drafts of articles.

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assisted in sampling, laboratory work, data analysis and article writing.

CONFLICT OF INTEREST

We would like to declare that we have no conflicts of interest related to this research.

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