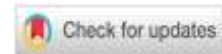




## The Phytochemical constituents and biological activities of sungkai (*Peronema canescens* Jack) leaves hydroethanolic extracts



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### Article Info

#### Article History:

Received 28 March 2024

Revised 04 April 2024

Accepted 07 April 2024

Published 30 April 2024

#### Keywords:

Antioxidant activity

*Peronema canescens*

Flavonoid

Phenolic

DNA protection



### ABSTRACT

*Peronema canescens*, Jack commonly known as "sungkai," has been traditionally used as a herbal medicine for various health conditions. This study aimed to explore the bioactive compound of the *P. canescens* leaves hydroethanolic extract, along with assessing its antioxidant and antimutagenic properties. Liquid chromatography-mass spectrometry (LC-MS) was utilized for phytochemical analysis of the hydroethanolic extracts, while antioxidant activity was evaluated through the DPPH radical scavenging method. Quantification of total phenolic and flavonoid content was achieved via colorimetric analysis. Furthermore, the DNA protection activity was assessed using plasmid pBR322 subjected to free radical treatment. The primary bioactive compounds identified in the *P. canescens* hydroethanolic extracts belonged to the alkaloid and flavonoid groups. The antioxidant activity of *P. canescens* leaves hydroethanolic extracts showed an IC<sub>50</sub> value of  $0.02 \pm 0.00$   $\mu\text{g/mL}$ . Additionally, the total flavonoid and phenolic content were measured at  $33,769 \pm 3,626$   $\mu\text{g QE/mL}$  and  $638,924 \pm 6,683$   $\mu\text{g GAE/mL}$ , respectively. Notably, *P. canescens* exhibited significant potential in mitigating DNA damage. In conclusion, the *P. canescens* leaves hydroethanolic extracts demonstrate promising attributes as a herbal medicine, highlighting notable antioxidant and antimutagenic effects.

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**Citation:** Rahayu, I., Sudrajat, S.E., Sancnia, Puspasari, M., & Timotius, K.H. (2024). Phytochemical constituents and biological activities of Sungkai (*Peronema canescens* Jack) leaves hydroethanolic extracts. *JPBIO (Jurnal Pendidikan Biologi)*, 9(1), 123-132. DOI: <https://doi.org/10.31932/jpbio.v9i1.3398>



## INTRODUCTION

*Peronema canescens*, Jack commonly known as sungkai or jati sabrang, is a wild plant from the *Verbenaceae* family. Sungkai is a significant export commodity, particularly in Sumatra and Kalimantan. This botanical species is utilized extensively in herbal medicine, with its entire plant being employed either through crushing or brewing for a range of medicinal purposes such as mouthwash, anti-parasitic treatment, relief for coughs and colds, among others (Brata and Wasih, 2021). Sungkai has been subject to analysis of its active constituents. This examination revealed flavonoids, alkaloids, steroids, phenolics, peronemin, sitosterol, isopropanol, phytol, diterpenoids, tannins, and saponins in Sungkai leaves. Notably, the compounds identified in Sungkai leaves exhibit primary activity as antioxidant and antibacterial (Ahmad & Ibrahim 2015).

Antioxidants are substances capable of counteracting the effects of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS). The antioxidants present in sungkai leaves aid in counteracting and reducing the impact of free radicals, thus protecting biological molecules against oxidative stress (Nimse & Pal 2015). Redox homeostasis denotes the equilibrium encompassing the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), along with their neutralization by antioxidants. This phenomenon interconnects with fundamental cellular processes, where oxidative stress ensues from an imbalance between pro-oxidants and antioxidant entities (Shadfar et al. 2023).

Oxidative stress can potentially trigger DNA damage and impair DNA repair mechanisms. Oxidative stress disrupts numerous cellular functions, notably those responsible for preserving DNA integrity. The accumulation of DNA damage may contribute to an increased mutation rate and can induce alterations in gene expression, ultimately leading to disruptions in cellular metabolism (Włodarczyk and Nowicka 2019). DNA damage was involved in the initiation of carcinogenesis and the development of degenerative diseases, such as obesity, diabetes mellitus, atherosclerosis, and cardiovascular disease. Notably, age-related neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease, increasingly manifest DNA damage and deficiencies in DNA repair mechanisms (Izzotti 2002; Shadfar et al. 2023).

Several studies have been conducted on phytochemical analysis, antioxidant activity, and immunomodulatory effects (Ahmad and Ibrahim 2015; Dillasamola et al. 2021), yet no investigation has been conducted into their potential in protecting DNA from damage. Consequently, this research aims to evaluate the active compounds present in polar extracts, their antioxidant activity, and DNA protection capabilities.

## RESEARCH METHODS

### Research Design

This research was a laboratory experimental study. LCMS was employed to analyze active compound content. Antioxidant activity was quantified relative to the existence of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). DNA protection activity was assessed using plasmid pBR322 and treated with the free radical.

### Population and Samples

*Peronema canescens* Jack (local name Sungkai) was taken from Palangkaraya, Kalimantan. One of the authors identified the plant utilizing the determination keys outlined by Maxwell (1978).

### Instruments

The LC-MS/MS analysis was conducted using the Waters LCMS/MS-QTOF system, utilizing the ToF MSE operational mode. A C18 column was utilized for this analysis. A



spectrophotometer was used to evaluate antioxidant activity, whereas an Electrophoresis apparatus was employed to assess DNA protection activity.

## Procedures

### 1. Plant extraction

Ten grams of sungkai powder underwent maceration with 80% ethanol in 300 mL of distilled water for a duration of 24 hours. The filtrate obtained from this process was subsequently evaporated, yielding a pellet. This pellet was then employed for phytochemical analysis, evaluation of antioxidant activity, and conducting a DNA protection assay.

### 2. LC-MS analysis

The bioactive constituents present in Sungkai hydroethanolic leaf extract were analyzed using LCMS/MS-QTQF (Waters), utilizing TOF MSE as the operational mode. The chromatographic separation was conducted using a C18 column, with the mobile phase consisting of 0.1% formic acid in acetonitrile and 0.1% formic acid in distilled water. Initially, 5% (w/v) samples in methanol were prepared and homogenized for 30 minutes. Subsequently, 10  $\mu$ L of the filtrate were injected into the system. The UNIFI software was utilized for the screening process to identify active constituents in the samples (Rahayu and Timotius 2022).

### 3. Total Phenolic Content

The total phenolic content was measured utilizing the Folin-Ciocalteu assay, with gallic acid as the standard. Specifically, 0.5 mL of the sample was combined with 2.5 mL of 10% Folin-Ciocalteu reagent. Incubation was done for 10 minutes. Then, 2.5 mL of 75 g/L Na<sub>2</sub>CO<sub>3</sub> was added to the sample-reagent mixture. This mixture was left to incubate for 2 hours at room temperature. Subsequently, absorbance measurement was performed at 765 nm. The total phenolic content was determined by quantifying it as gallic acid equivalent (GAE) utilizing a standard curve and subsequently expressed as mg GAE /mL (Shukla et al. 2016).

### 4. Total Flavonoid Content

Total flavonoid content was determined using the aluminum chloride colorimetric method. A standard curve was prepared by dissolving 50 mg of quercetin in 1 mL ethanol 95%, and subsequent dilution. The diluted standard solution (0.5 mL) was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% AlCl<sub>3</sub>.6H<sub>2</sub>O, 0.1 mL of 1M sodium acetate, and 2.8 mL of water. Following a 30-minute incubation period at room temperature, the absorbance of the solution was measured at a wavelength of 415 nm using a UV-Vis spectrophotometer. The acquired results were interpreted directly against the quercetin standard curve and expressed as mg EQ/mL (Chia-Chi et al. 2002).

### 5. Antioxidant Activity with DPPH Radical Scavenging

A volume of 500  $\mu$ L of various concentrations of hydroethanolic extract was reacted to 150 mM DPPH in absolute methanol, followed by an incubation process in the dark at room temperature for 30 minutes. An antioxidant standard, butylated hydroxytoluene (BHT), was employed as a reference substance. The absorbance of the mixture solution was measured at 517 nm (Singleton et al. 1999). The % free radical inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - \text{Abs Sample}}{\text{Abs control}} \times 100\%$$

### 6. DNA Protection Activity Assay

The DNA model utilized in this study was the pBR322 plasmid. The plasmid DNA pBR322 was treated with the OH<sup>•</sup> radical generated from the Fenton reaction. The conversion of plasmid DNA pBR322 from its supercoiled conformation to open-circular and linear forms was utilized as an indicator of DNA damage (Jeong et al. 2009). The reaction mixture (15  $\mu$ L)



consisted of 5  $\mu\text{L}$  of phosphate-buffered saline (PBS, 10 mM, pH 7.4), 1  $\mu\text{L}$  of plasmid DNA (0.5  $\mu\text{g}$ ), 5  $\mu\text{L}$  of the sample, 2  $\mu\text{L}$  of 1 mM  $\text{FeSO}_4$ , and 2  $\mu\text{L}$  of 1 mM  $\text{H}_2\text{O}_2$ . This mixture was then incubated at 37 °C for 30 minutes. Following the incubation period, 2  $\mu\text{L}$  of loading dye (Geneaid) was added to stop the reaction. Subsequently, the solution mixture was subjected to electrophoresis on a 0.85% agarose gel supplemented with 3  $\mu\text{L}$  of florosafe (Rahayu & Timotius, 2022).

### Data Analysis

The information acquired from LCMS comprised active compounds, which were then sorted into specific categories according to their corresponding compound groups. Descriptive data analysis was employed to provide a thorough summary of the data, abstaining from extensive statistical inferences. Linear regression was utilized to assess total phenolic content, total flavonoid content, and antioxidant activity. The analysis of DNA protection was conducted descriptively.

### RESULTS

Liquid chromatography-mass spectrometry (LC-MS) is an advanced analytical method that integrates the separation functionalities of liquid chromatography with the detection and characterization capabilities offered by mass spectrometry (Naczka and Shahidi, 2004). Two dominant compounds were identified in the hydroethanolic extract of Sungkai leaves: alkaloids and flavonoids. One alkaloid compound, betaine, was detected, while a total of 10 flavonoid compounds were identified, such as genkwanin, yuankanin, luteolin, sophorabioside, aceosidine, undulatoside A, and the derivatives of dimethoxyflavone, kaempferol, quercetin, and chromone (Table I).

**Table I.** LCMS Phytochemical analysis

No	Identified compound	Ionization mode	RT	MZ	Molecular formula	Response
Alkaloid						
1	Betaine	Positif	0,58	118 229 365	$\text{C}_5\text{H}_{11}\text{NO}_2$	13.860
Flavonoid						
2	3,5,6-Trihydroxy-4',7-Dimethoxyflavone	Positif	15,09	270 331 463	$\text{C}_{17}\text{H}_{14}\text{O}_7$	432.454
3	3,6,7-Trimethylquercetin	Positif	15,82	361 431 759	$\text{C}_{18}\text{H}_{16}\text{O}_8$	45.879
4	6-Methoxy-2-[2-(4'-methoxyphenyl)ethyl]chromone	Positif	16,48	327 367 711	$\text{C}_{20}\text{H}_{22}\text{O}_4$	208.719
5	Genkwanin	Positif	11,06	242 285 469	$\text{C}_{16}\text{H}_{12}\text{O}_5$	197.619
6	Kaempferol 3-O- $\alpha$ -L-rhamnopyranosyl-(1->2)- $\beta$ -D-glucuronopyranoside	Positif	12,46	301 317 617	$\text{C}_{27}\text{H}_{28}\text{O}_{11}$	22.009 6

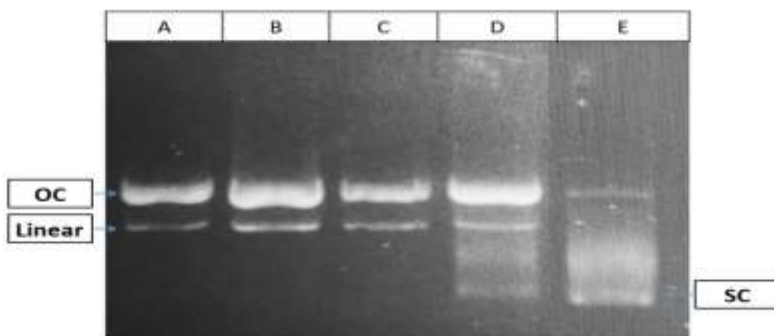


No	Identified compound	Ionization mode	RT	MZ	Molecular formula	Response
7	Kaempferol-3-gentiobioside	Positif	11,23	317 611 633	C27H30O1 6	154.508
8	Luteolin-7,4'-di-O- $\beta$ -D-glucopyranoside	Positif	11,41	317 611 633	C27H30O1 6	264.955
9	Quercetin-3-Oglucuronide 6''-methylester	Positif	11,17	315 493 625	C22H20O1 3	53.008
10	Yuankanin	Positif	10,46	285 579 601	C27H30O1 4	338.011
11	Sophorabioside	Positif	9,26	163 285 647	C27H30O1 4	68.836
12	Jaceosidin	Negatif	15,37	199 299 329	C17H14O7	130.995
13	Undulatoside A	Negatif	5,6	178 191 353	C16H18O9	10.103

The compounds constitute a group of potential antioxidants. This is demonstrated by the total phenolic and flavonoid contents and its ability to scavenge DPPH free radicals (Table 2). The research findings indicate that the antioxidant activity of Sungkai leaves hydroethanolic extract is stronger than that of BHT. This outcome is supported by its total phenolic and flavonoid contents. Phenolics and Flavonoids are commonly known for their antioxidant properties.

**Table 2.** Antioxidant activity of Sungkai leaves hydroethanolic extract

Sample	IC <sub>50</sub> ( $\mu$ g/mL)	Total Flavonoids Content ( $\mu$ gQE/mL)	Total Phenolic Content ( $\mu$ gGAE/mL)
Sungkai	0,02 $\pm$ 0,00	33,76 $\pm$ 3,62	638,92 $\pm$ 6,68
BHT	6,21 $\pm$ 0,21	-	-



**Figure 1.** DNA protection activity; The changes in the topological structure of DNA plasmid pBR322 in various concentrations.



We also evaluated its antioxidant capability in protecting DNA from free radicals. In this study, we employed the plasmid DNA pBR322 as a model. The plasmid DNA was subjected to OH<sup>-</sup> free radicals generated through the Fenton reaction. The results are evident from the changes in the plasmid DNA conformation (Figure 1). There is a difference in conformation between the normal plasmid DNA and those treated with free radicals. Administration of the sungkai leaves hydroethanolic extract restored DNA supercoil at 22,24 mg/mL. The results indicate that the antioxidant compounds of sungkai leave hydroethanolic extract have the potential to protect the DNA from free radicals.

**Table 3.** Antimutagenic analysis

Code	Treatment
A	Plasmid + H <sub>2</sub> O <sub>2</sub> + Fe <sub>2</sub> SO <sub>4</sub>
B	5,56 mg/mL Hydroethanol + Plasmid + H <sub>2</sub> O <sub>2</sub> + Fe <sub>2</sub> SO <sub>4</sub>
C	11,12 mg/mL Hydroethanol + Plasmid + H <sub>2</sub> O <sub>2</sub> + Fe <sub>2</sub> SO <sub>4</sub>
D	22,24 mg/mL Hydroethanol + Plasmid + H <sub>2</sub> O <sub>2</sub> + Fe <sub>2</sub> SO <sub>4</sub>
E	Non treated Plasmid

## DISCUSSION

This research indicates that the flavonoid group comprises the predominant active compounds found in the hydroethanolic extract of Sungkai leaves, while alkaloids were present in lesser quantities. Flavonoids and alkaloids are two classes of organic compounds commonly found in plants, each with distinct chemical structures and biological properties. Alkaloids are a diverse set of compounds originating from amino acids, displaying diverse biological activities, and can be synthesized by a range of organisms, including bacteria, fungi, and plants (Boratyński et al. 2019; Liu et al. 2019).

Alkaloids primarily exert immunomodulatory effects by regulating cytokines such as IL-6, IL-12, IL-1 $\alpha$ , TNF- $\alpha$ , IL-1 $\beta$ , and IL-10 (Liu et al. 2019). Betaine, also known as trimethylglycine, is a naturally occurring compound. It is derived from glycine by adding three methyl groups (Dobrijević et al. 2023). One of its primary functions is to modulate the levels of homocysteine in the bloodstream (McRae 2013). Homocysteine is associated with an increased risk of cardiovascular diseases, strokes, and various health issues when found in elevated concentrations. Betaine aids in the conversion of homocysteine into other beneficial compounds, thereby contributing to the maintenance of optimal levels of this amino acid in the bloodstream (Arumugam et al. 2021; Truit et al. 2021).

The flavonoid group identified in this study consists of luteolin, sophorabioside, jaceosidine, and derivatives of dimethoxyflavone, genkwanin, kaempferol, quercetin, and chromone. Flavonoids, which are polyphenolic compounds, significantly protect plant cells from microorganisms, insects, and UV radiation (Harborne & William, 2000). Flavonoids are synthesized via the phenylpropanoid metabolic pathway, forming diverse structural patterns within flavonoid subgroups. These structural differences account for the variations in the biological effects of these compounds on organisms (Santiago et al. 2021).

Flavonoids exhibit antioxidant, anti-inflammatory, and immunomodulatory properties (Serafini et al. 2010). Sophorabioside and undulatoside A are flavonoid glycosides. Sophorabioside has been reported to exhibit estrogenic activity, promote osteoblast proliferation, and inhibit IL-5 activity (Min et al. 1999; El Halawany et al. 2010; Xu et al. 2009). Sophorabioside is an anti-inflammatory agent by suppressing NF- $\kappa$ B signaling (Lee et al. 2013). Undulatoside A, recognized as a chromone derivative, is acknowledged for its anti-inflammatory, antimicrobial, and



immunomodulatory properties (Koz et al. 2009; Pereira et al. 2020; Yu et al. 2017). In silico studies have indicated its potential as an antiviral agent (Rahayu et al. 2022). Jaceosidin is a natural methylated trihydroxyflavone and has demonstrated antihyperglycemic, antioxidant, and anti-inflammatory characteristics (Han et al. 2018). Genkwanin is a methoxyflavone with antioxidant, anti-inflammatory, neuroprotective, anticancer, antidiabetic, and antiviral activities (El Menyiy et al. 2023). Luteolin, 3',4',5,7-tetrahydroxyflavone, demonstrates various biological effects, including anti-inflammatory, antiallergic, and anticancer properties (Lin et al. 2008). Kaempferol also suppresses the proliferation of cancer cells, hampers angiogenesis, and triggers apoptosis in cancer cells (Chen & Chen 2013). Quercetin is one of the flavonoid groups recognized for its anti-inflammatory, antihypertensive, vasodilatory, anti-obesity, antihypercholesterolemic, and antiatherosclerotic properties (David et al. 2016).

In this study, we also quantified the total phenolic and flavonoid content and its ability to scavenge the activity of the DPPH free radical (Table 2). The analysis revealed that the concentrations of flavonoids and phenolics were  $33,769 \pm 3,626 \mu\text{gGAE/mL}$  and  $638,924 \pm 6,683 \mu\text{gQE/mL}$ , respectively. The IC<sub>50</sub> of antioxidant activity was  $0,02 \pm 0,00 \mu\text{g/mL}$ , stronger than BHT (IC<sub>50</sub>  $6,21 \pm 0,21 \mu\text{g/mL}$ ). Sungkai leaves hydroethanolic extracts contain relatively high levels of total flavonoids and phenolics and exhibit potent antioxidant activity. Flavonoids and phenolics play a crucial role in antioxidant activity, wherein flavonoids are categorized within the phenolic compound group and exhibit a direct correlation with antioxidant effectiveness (Panche *et al.* 2016). Phenolic compounds contribute to antioxidant efficacy owing to their inherent structural characteristics (Munteanu & Apetrei 2021). This contribution manifests through various mechanisms, including reduction processes, capture of free radicals, chelation of metals, quenching of singlet oxygen, and donating electrons (Phaniendra et al. 2014). The findings highlight a direct association between antioxidant activity and the levels of total phenolics and flavonoids. The higher the levels of flavonoids and phenolics, the stronger the antioxidant activity of Sungkai leaves hydroethanolic extracts.

We assessed the antioxidant potency through its activity in protecting DNA from damage caused by free radicals. Free radicals are known to induce DNA damage, resulting in mutagenesis. It is closely associated with the onset of diseases (Izzotti, 2002; Shadfar et al. 2023). In this study, the DNA model utilized was plasmid pBR322. The hydroxyl radicals (OH<sup>-</sup>) generated through the Fenton reaction are responsible for instigating damage to the DNA strand, thereby causing a conversion from its typical supercoiled (SC) structure to an open circular (OC) conformation. Figure 1 demonstrates the potency of the Sungkai leaves hydroethanolic extract in protecting DNA. When DNA damage occurs, the regular conformation of the plasmid changes. The typical conformation of plasmid DNA consists of three forms: Open Circular (OC), Linear (L), and Supercoiled (SC) (Figure 1). DNA damage becomes apparent when there is a high intensity of OC and the absence of SC form. The research findings indicate that at a concentration of 22.24 mg/mL, the hydroethanolic extract of Sungkai leaves can restore the typical conformation of plasmid DNA (Figure 1).

DNA damage is recognized as a crucial determinant in cancer development. When DNA undergoes damage, it generates abnormal nucleotides, disrupting one or both DNA strands. The interruption of a DNA strand precipitates various intricate issues, including mutations and genome instability (Alhmod et al. 2020). Consequently, when DNA sustains damage, genotoxic agents interfere with the covalent bonds between nucleotides, impeding normal replication and transcription processes within the genome (Cannan & Pederson, 2016). Although the DNA repair mechanism can rectify damaged DNA, its efficacy is not absolute and comprehensive. The outcomes of this repair mechanism may give rise to additional complications, such as chromosome aberrations and mutations that impede cellular functions (Sáez, 2018). Defects in genes can

manifest in oncogenes, tumor suppressor genes, and genes governing the cell cycle, which prove detrimental to cellular defense and proliferation. Such DNA damage is commonly referred to as carcinogenic (Basu, 2018).

Conversely, DNA damage contributes to the aging process through cumulative accumulation over time. At the cellular level, the ability to maintain balance diminishes as cells become unable to rectify protein distortions stemming from DNA damage. Consequently, this can disrupt the normal functioning of cells, thereby predisposing individuals to various diseases, including Alzheimer's. In Alzheimer's patients, cellular dysfunction arises due to the inability to produce adequately folded proteins, leading to the formation of toxic protein aggregates. Individuals with diminished DNA damage repair capabilities face an elevated risk of developing cancer as they age (Luu & Palczewski, 2018).

Therefore, it appears that the antioxidant compounds found in the Sungkai leaves' hydroethanolic extract may protect DNA from damage. Their activity is evident through their ability to scavenge free radicals and reduce damage effects. The hydroethanolic extract of Sungkai leaves has the potential to safeguard genomic stability, thus reducing mutations and the development of diseases.

## CONCLUSION

In this study, we discovered that the hydroethanolic extracts of Sungkai leaves contain two main categories of compounds: alkaloids and flavonoids. The antioxidant compounds exhibit higher activity than BHT and demonstrate protective activity against DNA damage caused by free radicals. Further research is needed to investigate the activity of active compounds in preventing the progression of degenerative diseases.

## ACKNOWLEDGMENT

We are grateful to the Research and Community Institute of Universitas Kristen Krida Wacana (LPPM-UKRIDA) for funding this research.

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