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# Antioxidant, Antibacterial, Total Phenolic and Flavonoid Contents of Sungkai Leaves (*Paronema canescens*)

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ABSTRACT

The leaves of the sungkai (*Paronema canescens*) have been used as a traditional medicine to treat various diseases. The present study determined the antioxidant, antibacterial, total phenolic content, and flavonoid content of extracts of *P. canescens* leaves. Fresh leaves of *P. canescens* was extracted by maceration using solvents of increasing polarity (*n*-hexane, ethyl acetate, and methanol). Each extract was tested for antioxidant activity using the DPPH method, antibacterial activity was tested using the disc diffusion method, analysis of the total phenolic content was done using the Folin–Ciocâlteu reagent, and total flavonoid using the aluminium chloride colorimetric method. The study showed that the ethyl acetate extract had higher antioxidant activity than the other extracts with an $IC_{50}$ of 320 µg/mL. The antibacterial activity test had a minimum inhibitory concentration (MIC) of 62.5 µg/mL against *E. coli* and *S. aureus* for all the extracts, but only the *n*-hexane extract showed MIC of 62.5 µg/mL against *S. typhi*. Betulinic acid from the *n*-hexane extract also showed antibacterial activity with MIC of 62.5 µg/mL. In the analysis of total phenolic and flavonoids, the ethyl acetate extract had higher values than the other extracts, with values of 68.71 ± 0.17 mg Gallic Acid Equivalent (GAE)/g and 2.29 ± 0.05 mg Quercetin Equivalent (QE)/g for total phenol and flavonoid content, respectively. It can be concluded that sungkai leaves showed moderate antioxidant activity, moderate antibacterial activity, and much lower flavonoid content than phenolic content.

***Keywords***: Antioxidant, Antibacterial, Phenolic, Flavonoid, *Paronema canescens*.

## Introduction

Indonesia is a tropical country with high biodiversity, so it is one of the potential countries for obtaining new bioactive compounds. Research on the search for bioactive compounds from traditional medicinal plants is growing, in line with ethnobotanical surveys of various ethnicities, especially in Indonesia. The survey reported that some plants have been used for treatment of diseases by the local community. Scientific assessment is needed for these medicinal plants. Additionally, many freely-sold herbal products attracts public interest for disease treatment due to their affordability, perceived efficacy and better tolerance compared to modern medicine.[1] However, some of these herbal products do not have well-documented scientific information. One of the herbs of traditional medicine is sungkai (*Paronema canescens*). Indonesian people traditionally use *P. canescens* to treat diseases, such as toothache,[2] fever,[3] stomach ache, skincare, after childbirth,[4] and malaria.[5] Its use as a medicine is especially prevalent in South Sumatra, where the community has additionally used sungkai leaves to treat warts,[6] and hypertension.[7]
Based on literature studies, some scientific information about the chemical content and biological activity of the *P. canencens* plant has been reported. The phytochemical test of an ethanol extract of the sungkai plant was positive for steroidal, triterpenoid and phenolic compounds.[8] The compounds of these phenolic groups are antioxidant and have various biological activities, such as antibacterial, antidiabetic, antidiabetic, anticancer, antihypertensive and anti-hyperlipidemia. The methanol extract of the *P. canencens* leaves was reported to have antibacterial activity against *S. mutans*, *S. thyposa*, *B. subtilis*, and *S. aureus*.[4] In another study, antimalarial compounds was found in the acetone extracts of *P. canescens* leaves.[9] It is also reported that the sungkai leaf extract was useful as an insecticide against the larvae of *Plusia* sp. The *n*-hexane and ethyl acetate extract of the *P. canescens* stem bark had an antioxidant activity with $IC_{50}$ in the *n*-hexane extract 44.55 µg/mL and in the ethyl acetate extract of 43.67 µg/mL.[10] In this study, we reported antioxidant and antibacterial activities of the fractions from *P. canescens* leaves.

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## Materials and Methods

*Sample collection*

The fresh leaves of *P. canescens* was collected from the Musi Banyuasin Regency of South Sumatera, Indonesia, in October 2019. The plant was identified as *P. canescens* by Dr Laila Hanum, head of the botany laboratory, University of Sriwijaya. A voucher specimen has been deposited at the Botany Laboratory in the Biology Department at the University of Sriwijaya with Voucher specimen VIC 2704.

*Extraction process*

The fresh leaves of *P. canescens* (1.2 kg) was extracted using the maceration method with step gradient polarity using the solvents *n*-hexane, ethyl acetate, and methanol, followed by filtration. This process (of soaking and filtration) was repeated three times.[11] The filtrate was evaporated at 60°C to obtain concentrated extracts. The concentrated extracts were weighed and the percentage yields calculated.

*Determination of antioxidant activity*

The antioxidant activity of the extracts was evaluated using the DPPH method according to a previous method of Xu *et al*[12] with some modifications. Extracts were made in series of different



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concentrations i.e. 50; 100; 200; 300; 400; 500; and 1000 μg/mL in DMSO. From each concentration, 200 μL was taken into a dark bottle containing 3.8 mL of 0.05 mM DPPH. The mixture was vortexed for 2 min, then incubated at room temperature for 30 min in the dark. The study was conducted three times. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as a positive control, methanol used as blank (4 mL), and DPPH (3.8 mL) was added to 200 μL of DMSO, which act as the negative control. The percentage of inhibition was calculated using the formula: % Inhibition

$$= \frac{\text{Absorbance of Control} - \text{Absorbance of sample}}{\text{absorbance Control}} \times 100$$

Based on the data, a linear regression equation curve was plotted between the sample concentration and the percent inhibition of the sample to determine the $IC_{50}$ value.[13]

*Analysis of total phenolic*

The extracts total phenolic contents were determined using the Folin Ciocalteu reagent, following the method described by candra *et al*[14] with slight modifications. Analysis using a spectrophotometric method and gallic acid was used as standard. 1.0 mL sample was added to 2.5 mL of Folin reagent after 5 minutes, 10 mL of 7.5% $Na_2CO_3$, incubated for 90 min at room temperature, then the absorbance was measured at $\lambda_{max}$ 760 nm. A standard curve of gallic acid was made with concentrations of 18; 20; 23; 25; 27; 30 µg/mL.

*Determination of total flavonoid content*

The total flavonoid content was determined by spectrophotometry based on reaction with $AlCl_3$ following the method described by Amalitc *et al*[15] with slight modifications. Sample extract 1 mL was added to 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$, it was mixed and left for 5 min. Into this mixture, 0.3 mL of 10% $AlCl_3$ and 2 mL of 1.0 M NaOH was added and homogenized for 5 min. Quercetin standard curves were made with a concentration series of 100; 80; 60; 40, and 20 μg/mL and treated the same as the samples described above. Absorbance fractions and standards were measured at $\lambda_{max}$ 510 nm. The blanks are defined as all reagents used without quercetin or sample, and the measurements were replicated thrice.

*Antibacterial activity test*

The antibacterial screening was carried out by the disc diffusion method using paper disc with a diameter of 6 mm. The sample concentrations used in the antibacterial activity test were 4%, 2%, 1% and 0.5%. Disc paper was dipped at each sample concentration, then placed on nutrient agar (NA) media that had been inoculated with *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *S. typhi* (ATCC 19214) bacteria. Incubation at 37 °C for 24 hours. Observations were made based on the formation of an inhibition zone around the disc paper.[16]

*Determination MIC*

The minimal inhibitory concentrations (MIC) determined by the micro-dilution method were conducted according to the Clinical and Laboratory Standards Institute[17] with slight modifications. Liquid culture (30 µL) from each bacterium was inoculated into a plate that already contained 180 µL (hole 1) and 100 µL (hole 2, etc.) NB medium and stirred. The isolated compounds were prepared with concentrations of 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.9, 0.97, 0.48 and 0.24 µg / mL bacterial samples that were made in various concentrations were inserted into the plate. Plate holes 11 and 12 were used for a DMSO negative control and tetracycline (10 mg/mL concentration) positive control. The cultures were incubated for 24 hours at 37°C. Antibacterial activity was observed by the formation of a clear solution.[18]

*Statistical analysis*

Measurements were made in triplicate. Data are provided as mean ± SD. Data were analyzed statistically using ANOVA (α 0.05), followed by the Duncan New Multiple Range Test (DNMRT) at 0.05.

## Results and Discussion

Fresh sungkai leaves (1.2 kg) were extracted by maceration via solvent with increasing polarity in triplicate. After being concentrated, the *n*-hexane extract (15.97 g) was obtained, ethyl acetate extract (62.69 g), and the methanol extract (56.40 g), with yields of 1.33%, 5.22%, and 4.70%, respectively. The product of the ethyl acetate extract was higher than the other extracts. The higher yield of ethyl acetate extract implied that the leaves of *P. canescens* contained more compounds with high semi-polar property rather than non-polar and polar property. Besides that, the higher product is presumably because ethyl acetate is a semi-polar solvent that can dissolve both non-polar and polar compounds.[19]

*Antioxidant activity*

The antioxidant activity of the fractions was determined using DPPH method. Absorbance was recorded at 517 nm. The antioxidant activity of each extract was expressed as a percentage of inhibition, as shown in Table 1. Percentage inhibition implies the number of DPPH radicals absorbed by the test sample. The higher the percentage inhibition, the more active the test sample.[20] Table 1 shows that the higher the test concentration, the greater the inhibition value. At the same concentration (1000 μg/mL), the percentage inhibition of ethyl acetate extract was 76.14 ± 6.20. It was higher than other extracts, while ascorbic acid as a standard at concentration 100 μg/mL showed the percentage inhibition of 95.19 ± 0.51 (Table 2). Based on the statistical analysis of the methanol extract and *n*-hexane extract, the percentage inhibitions were not significantly different ($p > 0.05$) but were significantly different from the ethyl acetate extract ($p < 0.05$). The antioxidant activity was influenced by the flavonoid and phenolic contents in the extract. The Flavonoids will donate hydrogen atoms or electrons to free radicals to stabilize radical compounds so that the higher the flavonoid content in the extract, the higher the antioxidant activity.[21] The total flavonoid content has an influence on antioxidant activity.[12]

Based on the inhibition percentage, the $IC_{50}$ was determined i.e. DPPH reduced by 50% upon extract addition. The $IC_{50}$ value determination is based on the linear regression curve for the relationship between concentration (x) and the percent inhibition value (y). The data in Table 3 show that the ethyl acetate extract had the smallest $IC_{50}$ value compared to other extracts (320 μg/mL) and ascorbic acid as a standard (10.69 μg/mL). The smaller the $IC_{50}$ value, the more active the fraction. An extract is categorized as (potentially) a strong antioxidant if it has an $IC_{50}$ value of < 200 μg/mL, moderate if between 200 and 1000 µg/mL and inactive if > 1000 µg/mL.[22] Based on the data obtained, only the ethyl acetate extract was classified as a moderate antioxidant, while the other two extracts were inactive.

The extract belonging to the moderately active category of antioxidants still can be used as a source of antioxidant because sometimes the compounds in pure form are more active than in their extract form. Based on the literature, Rosdiana[10] reported that the *n*-hexane and ethyl acetate extracts of *P. canescens* stem bark had bioactivity as an antioxidant with an $IC_{50}$ *n*-hexane extract of 44.55 μg/mL and ethyl acetate extract of 43.67 μg/mL. In the leaves of sungkai, the target of this study, the $IC_{50}$ value was much greater. This indicates that the antioxidant activity of the sungkai stems is much stronger than that of the leaves.

*Total phenolic and flavonoid content*

Determination of the total amount of phenols was carried out in each extract by using spectrophotometry and the Folin–Ciocâlteu reagent. The total phenolic value of each extract was determined from the linear regression of the standard curve of gallic acid. The total phenolic value of the sample (mg GAE / g), is shown in Table 4. The ethyl acetate extract shows the highest total phenolic content of 68.71 ± 0.17 mg GAE/g than the other extracts.

Determination of total flavonoids by colorimetry was based on the formation of a flavonoid–$AlCl_3$ complex. The total flavonoid content (TFC) of the extract was determined from the regression equation of the quercetin calibration curve and the flavonoid value in mg quercetin to dry weight of the sample (mg QE/g). The highest level of


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flavonoids was indicated by the ethyl acetate extract compared to the other extracts with a total flavonoid value of 2.29 ± 0.05 mg QE/g. The highest value of total polyphenol and total flavonoid obtained from the fresh sarcocarp of *C. multiflorus* was extracted by ethyl acetate.[23] The amount of total polyphenol and total flavonoid is significant, which might indicate that most of the polyphenols and flavonoids were more soluble in a less polar solvent, such as ethyl acetate.[23] This finding confirmed that the polyphenol and flavonoid are more soluble in a semi-polar solvent such as ethyl acetate.

The data in Table 4 also show that the total phenolic content was much higher than the flavonoid content in every extract. The higher the phenolic fraction content, the higher the total flavonoid levels. Flavonoids include phenolic compounds in addition to other compound groups. Data in Table 3 show that the low levels of flavonoids from each extract were deficient. Based on literature studies, the levels of flavonoids in the sungkai leaf extracts were deficient. Agbo *et al*[24] reported 3 samples from parts of medicinal plants from Nigeria and result showed that *E. prostrate*, *P. bifurcatum* and *A. platycaulon* had high total phenolic content (97.77 ± 0.77, 87.62 ± 1.22 and 82.33 ± 0.30 mg GAE/g) and *P. bifurcatum* had the high flavonoid content (648.67 ± 12.3 mg QE/g). The ratio of flavonoids to total phenolic content was determined as the fraction that contains the most flavonoid compounds. The data showed that the three extracts gave a ratio of < 1, indicating a low level of flavonoids. Flavonoids are known to have various biological effects, including antibacterial, antioxidant, antidiabetic, antihypertensive, anti-tumour, anticancer activity, among others. The high phenolic and flavonoid contents in an extract usually indicates high antioxidant activity. Kristinaningsih *et al*[25] reported similar results of phytochemical screening of phenolics with antioxidant activity value exhibited by ethanol extract of *Aleurites moluccana* leaves. Our result concludes that low levels of flavonoids correspond to low antioxidant activity of each extract.

*Antibacterial activity*

The activity value is expressed as the inhibition zone diameter (clear zone). The anti-bacterial screening was carried out on the three extracts, as shown in Table 5. The results showed that the antibacterial activity of all extracts had an inhibition zone diameter ranging from 7.7 ± 0.1–9.1 ± 0.4 mm at test concentrations of 500 µg/mL to 4000 µg/mL. DMSO, the negative control, had no inhibition zone. The antibacterial properties of the extract of *P. canescens* are also thought to be related to the chemical content of flavonoids and phenolics in the extract.[26] In general, the diameter of the inhibition zone is greater if the concentration is higher. However, in several test concentrations, it was seen that the difference in concentration did not have a significant difference in inhibition zone diameter values ($p > 0.05$). The antibacterial activity of the methanol extract was lower than ethyl acetate and *n*-hexane extracts; however, the *n*-hexane and ethyl acetate extracts' inhibition zone diameter was not significantly different. The data in Table 5 also show that the extracts of methanol, ethyl acetate and *n*-hexane had an antibacterial activity that was not significantly different ($p > 0.05$) against the three tested bacteria. Based on the literature, antibacterial activity was classified into strong activity if the inhibition zone diameter was 10–20 mm, moderate activity if 5–10 resistance to the three tested bacteria up to a test concentration of 500 µg/mL.

The result of the MIC value is shown in Table 6. The results showed that all extracts gave MIC value of 62.5 µg/mL for *E. coli* and *S. aureus*, for the *S. typhi* bacteria, only the n-hexane extract gave MIC value of 62.5 µg/mL while the other extracts gave MIC value of 125 µg/mL. The extract is stated as antibacterial if it gives MIC value <100 µg/mL.[28] Based on this data, the three extracts are categorized as active against *E. coli* and *S. aureus* with MIC value of 62.5 µg/mL while against *S. typhi*, only n-hexane extract showed antibacterial activity. Extracts obtained from ethyl acetate and methanol showed weak activity with MIC value of 125 µg/mL. mm, and weak if it had an inhibition zone diameter of < 5 mm. Based on this criterion, all the extracts of *P. canestens* leaves have moderate antibacterial activity against all bacteria tested.

**Table 1:** The influence of the sample's concentration on DPPH inhibition of leaves fractions methanol, ethyl acetate, and n-hexane *P. canescens*

| Concentration (µg/mL) | Inhibition percentage (% I) ± SD | | |
|---|---|---|---|
| | n-Hexane | Ethyl acetate | Methanol |
| 50 | 24.69 ± 0.25$^e$ | 28.13 ± 4.51 | 15.95 ± 1.65$^g$ |
| 100 | 25.30 ± 0.38$^e$ | 43.86 ± 2.56 | 19.41 ± 0.14$^f$ |
| 200 | 25.92 ± 0.07$^e$ | 47.42 ± 3.19 | 24.81 ± 0.28$^e$ |
| 300 | 27.64 ± 0.77$^d$ | 50.73 ± 1.44 | 27.40 ± 0.60$^d$ |
| 400 | 28.50 ± 0.18$^d$ | 53.80 ± 7.65 | 28.13 ± 0.33$^d$ |
| 500 | 31.33 ± 1.28$^c$ | 63.27 ± 3.14 | 28.87 ± 0.87$^d$ |
| 1000 | 40.90 ± 3.40$^a$ | 76.14 ± 6.20$^b$ | 42.26 ± 1.96$^a$ |

Numbers followed by the same subscript indicate not significantly different according to Duncan New Multiple Range Test (DNMRT) 5%.; The experiments were repeated at three times, SD: Standard deviation.

**Table 2:** The influence of standard ascorbic acid concentration on DPPH inhibition

| Concentration (µg/mL) | % inhibisi |
|---|---|
| 6.25 | 40.1 0 ± 1.32$^a$ |
| 12.5 | 52.16 ± 0.41$^b$ |
| 25 | 75.05 ± 1.23$^c$ |
| 50 | 93.15 ± 0.61$^d$ |
| 100 | 95.19 ± 0.51$^d$ |

Numbers followed by the same subscript indicate not significantly different,The experiments were repeated at three times, SD: Standard deviation

**Table 3:** Inhibition concentrations of 50% of DPPH

| Fraction | $IC_{50}$ (µg/mL) | Antioxidant category * |
|---|---|---|
| n-Hexane | 1567 | Inactive |
| Ethyl acetate | 320 | moderate active |
| Methanol | 1281 | Inactive |
| Standard Ascorbic acid | 10.69 | Strong active |

*strong: IC value < 200 µg/mL, moderate $IC_{50}$ : 200 and 1000 µg/mL and inactive $IC_{50}$ : > 1000 µg/mL.

**Table 4:** Total phenolic and flavonoids contents of fractions *P. canescens*

| Fractions | Total Phenolic (mg GAE/g) | Flavonoid (mg QE/g) | Ratio F/P |
|---|---|---|---|
| n-Hexane | 9.28 ± 0.08 | 0.77 ± 0.13 | 0.083 |
| Ethyl acetate | 68.71 ± 0.17 | 2.29 ± 0.05 | 0.033 |
| Methanol | 24.30 ± 0.17 | 1.92 ± 0.03 | 0.079 |

The experiments were repeated at three times, SD: Standard deviation, F: Flavonoid, P: Phenolic.



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**Table 5:** Antibacterial activity of fractions the leaves of *P.canescens*

| Fractions | Concentration ($\mu$g/mL) | Zone of inhibition (mm) ± SD | | |
|---|---|---|---|---|
| | | ***E. coli*** | ***S. aureus*** | ***S. typhi*** |
| Methanol | 4000 | 8.6 ± 0.1 | 8.8 ± 0.3 | 10.1± 0.6 |
| | 2000 | 8.3 ± 0.5 | $8.6 \pm 0.2^{a}$ | 9.2 ± 0.2 |
| | 1000 | 8.1 ± 0.2 | $8.5 \pm 0.1^{a}$ | 7.9 ± 0.9 |
| | 500 | $7.7 \pm 0.1^{b}$ | $7.9 \pm 1.1^{b}$ | 7.3 ± 0.2 |
| Ethyl acetate | 4000 | $8.9 \pm 0.1^{d}$ | $8.0 \pm 0.8^{c}$ | 8.7± 0.1 |
| | 2000 | $8.8 \pm 0.1^{d}$ | $7.9 \pm 0.4^{c}$ | 8.1± 0.5 |
| | 1000 | 8.6 ± 0.6 | 7.7 ± 0.5 | 7.5 ± 0.4 |
| | 500 | 8.0 ± 0.4 | 7.6 ± 0.1 | 7.1± 0.1 |
| Hexane | 4000 | $9.1 \pm 0.4^{d}$ | 8.8 ± 0.4 | 9.2 ± 0.8 |
| | 2000 | $8.9 \pm 0.3^{d}$ | 8.5 ± 0.3 | 8.7 ± 0.2 |
| | 1000 | 8.2 ± 0.5 | 8.3 ± 0.2 | 8.2 ± 0.1 |
| | 500 | 7.8 ± 0.3 | 8.1 ± 0.2 | 7.9 ±0.6 |
| Control | | NI* | NI* | NI* |

Control: 10 % DMSO, NI* : No inhibition Numbers followed by the same subscript indicate not significantly different according to Duncan New Multiple Range Test (DNMRT) 5%.; Experiments were repeated at least three times. Data are presented as Mean ± SD, SD: Standart deviation.

The methanol extract of the *P. canescens* leaves was reported to have antibacterial activity against *S. mutans*, *S. thyposa*, *B.subtilis*, and *S. aureus* with MIC of 10000 µg/mL for *S. mutans*, an MIC of 15000 µg/mL for *S. thyposa* and *B. subtilis*, and 20000 µg/mL MIC for *S.aureus*.[5] In this study, it was found that all extracts still provided Separation and purification of the n-hexane extract using column chromatographic techniques was able to obtain pure compounds, which was identified as betulinic acid.[29] The antibacterial activity test of the isolated compound is shown in Table 6. The MIC values for all three extracts against the three bacteria are presented in Table 6. The results showed that all fractions had a MIC of 62.5 µg/mL for *E. coli* and *S. aureus*. For the *S. typhi* bacterium, only the *n*-hexane extract had MIC value of 62.5 µg/mL; the others had MIC value 125 µg/mL. In Table 7, it was shown that the inhibition zone diameter increases with concentration of the test compound. Statistical analysis showed that the values were not significantly different at different concentrations of the inhibition zone ($p > 0.05$). The isolated compound has antibacterial activity with MIC of 125 $\mu$g/mL with an inhibition zone diameter of 7.5 ± 0.1–8.2 ± 1.2 mm against the three tested bacteria. Statistical analysis showed no significant difference among the bacteria ($p > 0.05$). The antibacterial activity of the pure compound was compared to the *n*-hexane extract. The antibacterial activity of the isolated compound was observed to be higher than that of the extract. This shows that the compound is more active in its pure form than when present in an extract. The MIC of the pure compound was determined by the dilution method (Table 8).

The MIC value was determined based on the formation of a clear solution. Table 8 shows that at concentration of 62.5 $\mu$g/mL, a clear solution was formed for all tested bacteria, while at a concentration of 31.25 $\mu$g/mL, a cloudy solution was formed. It was concluded that the isolated compound had a MIC value of 62.5 $\mu$g/mL against the three tested bacteria (*E. coli*, *S. aureus*, and *S. typhi*).

**Table 6:** Determination MIC value of fractions leaves of *P. canescens*

| Fractions | Bacterial | Concentrations ($\mu$g/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 125 | 62.5 | 31.25 | 15.62 | 7.8 | 3.90 | 1.95 | 0.97 |
| Methanol | *E. coli* | + | + | - | - | - | - | - | - |
| | *S. aureus* | + | + | - | - | - | - | - | - |
| | *S. typhi* | + | - | - | - | - | - | - | - |
| Ethyl acetate | *E. coli* | + | + | - | - | - | - | - | - |
| | *S. aureus* | + | + | - | - | - | - | - | - |
| | *S. typhi* | + | - | - | - | - | - | - | - |
| n-Hexane | *E. coli* | + | + | - | - | - | - | - | - |
| | *S. aureus* | + | + | - | - | - | - | - | - |
| | *S. typhi* | + | + | - | - | - | - | - | - |

+: clear -: blurry

**Table 7:** Antibacterial activity of betulinic acid from the n-hexane fraction leaves of *P.canescens*

| Sample | Concentration (µg/mL) | Zone of inhibition (mm) ± SD | | |
|---|---|---|---|---|
| | | ***E. coli*** | ***S. aureus*** | ***S. typhi*** |
| | 1000 | $10.3 \pm 1.3^{b}$ | $9.8 \pm 1.1^{d}$ | $9.8 \pm 0.3^{d}$ |
| Betulinic acid | 500 | $10.1 \pm 1.6^{b}$ | $9.2 \pm 1.4^{d}$ | $8.9 \pm 0.1^{c}$ |
| | 250 | $7.8 \pm 0.1^{a}$ | $8.8 \pm 0.8^{c}$ | $8.4 \pm 0.1^{c}$ |
| | 125 | $7.5 \pm 0.1^{a}$ | $8.7 \pm 1.9^{c}$ | $8.2 \pm 1.2^{c}$ |

Numbers followed by the same subscript indicate not significantly different according to Duncan New Multiple Range Test (DNMRT) 5%.; Experiments were repeated at least three times. Data are presented as Mean ± SD, SD: Standart deviation


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**Table 8:** Determination MIC value of betulinic acid

| Sample | Bacterial | Concentrations (µg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **125** | **62.5** | **31.25** | **15.62** | **7.8** | **3.90** | **1.95** | **0.97** |
| Betulinic acid | *E. coli* | + | + | - | - | - | - | - | - |
| | *S. aureus* | + | + | - | - | - | - | - | - |
| | *S. typhi* | + | + | - | - | - | - | - | - |

+: clear -: blurry

## Conclusion

All extracts of the leaves from sungkai (*P. canescens*) show weak antioxidant activity and moderate antibacterial activity. The sungkai leaves also have much lower levels of flavonoids than phenolics. Because of the complexity of natural phytochemicals in the extract, pure compound was also assessed for the overall antioxidant and antibacterial potential of *P. canescens* leaves.

## Conflict of interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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# Antioxidant, Antibacterial, Total Phenolic and Flavonoid Contents of Sungkai Leaves (Paronema canescens)

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