Determination of Flavonoid Content and Anti-Inflammatory Activity 
Extract and Fraction of Sungkai Leaf (Peronema canescens Jack)

Penentuan Kadar Flavonoid dan Aktivitas Anti-Inflamasi 
Ekstrak dan Fraksi Daun Sungkai (Peronema canescens Jack)

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Abstract: The SARS-CoV-2 virus is the infectious agent that causes COVID-19, a feverish condition 
brought on by inflammation in the infected patient’s body. Sungkai leaf (Peronema canescens Jack) 
is one of the Indonesian people who rely on herbal remedies to treat COVID-19. This study aimed to 
determine how much flavonoids are present in ethanol, ethyl acetate, and n-hexane extracts, as well as 
the anti-inflammatory properties of ethanol extract, ethanol fraction, and n-hexane fraction of Sungkai 
leaves. The procedure was performed in vitro with a UV-visible spectrophotometer by observing the 
absorption response to inhibition of denaturation of inflammatory protein. The inhibition value was then 
computed via linear regression, and the IC50 and IC70 values were ascertained afterward. The ethanol 
fraction, ethanol extract, and n-hexane fraction in this investigation had the best inhibition values (%) at 
a concentration of 15 ppm, corresponding to 74.27%, 54.48%, and 18.52%. Comparatively, the ethanol 
extract contained the largest amounts of flavonoids, 38.782 µg/mL. The sungkai leaves ethanol fraction 
>n-hexane fraction>ethanol extract had the best IC50 and IC70 values.

Keywords: COVID-19, inflammation, infectious disease, protein denaturation, SARS-CoV-2 virus.

Abstrak : Virus SARS-CoV-2 menyebabkan penyakit COVID-19, yang menyebabkan demam dan per-
adangan di tubuh pasien. Daun sungkai (Peronema canescens Jack) adalah salah satu tanaman herbal 
yang diyakini masyarakat Indonesia dapat mengatasi COVID-19. Tujuan dari penelitian ini adalah untuk 
mengetahui kadar flavonoid pada ekstrak (etanol, etil asetat, dan n-heksan) dan sifat antiinflamasi ekstrak 
etanol, fraksi etanol, dan fraksi n-heksan daun sungkai. Metode ini dilakukan secara in vitro dengan 
 menggunakkan spektrofotometer UV-Vis untuk melihat respon serapan terhadap penghambatan denaturasi 
protein penyebab inflamasi. Selanjutnya, nilai inhibisi (%) terbaik ditemukan pada konsentrasi 15 ppm, 
yaitu fraksi etanol, ekstrak etanol, dan fraksi n-heksan sebesar 74,27% dan 54,4 persen. Ekstrak etanol daun 
sungkai memiliki kadar flavonoid tertinggi sebesar 38.782 g/mL. Nilai IC50 dan IC70 dari daun Sungkai 
secara berurutan adalah fraksi etanol, fraksi n-heksan, dan ekstrak etanol.

Kata kunci : COVID-19, denaturasi protein, inflamasi, penyakit infeksi, virus SARS-CoV2.

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INTRODUCTION

SEVERE acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the viral disease known as Coronavirus Disease 19 (COVID-19), a major global health concern. The most prevalent complaints from COVID-19 patients are fever (98.6%), exhaustion (69.6%), dry cough (59.4%), and shortness of breath (31.2%)\(^{(3)}\). Fever is one of the most straightforward indicators to show the occurrence of an inflammatory process in sufferers of COVID-19. Inflammation is a mechanism of the body to deal with chemicals that can cause damage to body tissues and the body’s efforts to restore tissue when injured\(^{(3)}\). Causes of inflammation include physical agents, immunologic reactions, infection with pathogenic organisms, and protein denaturation\(^{(3)}\). Because it can result from external pressure or substances like strong acids or bases, concentrated inorganic salts like organic solvents (alcohol or chloroform), heat, or both, protein denaturation is a cause of inflammation\(^{(4,5)}\). Denatured proteins in living cells interfere with cell function and may lead to cell death. Drugs that reduce inflammation can be made from substances that prevent protein denaturation\(^{(5,6)}\).

Anti-inflammatory drugs are a class of drugs that have activity to suppress or reduce inflammation\(^{(6)}\). NSAIDs, or non-steroidal anti-inflammatory drugs, are modern medications that are frequently used as anti-inflammatories. NSAIDs typically cause stomach ulcers as a side effect. So it is necessary to look for alternative treatments to control and combat pain and inflammation with relatively minor side effects, for example, drugs derived from plants or natural materials\(^{(7)}\).

The Sungkai plant is native to Indonesia and can be found in South Sumatra and Kalimantan. Empirically, and usually in South Sumatra, Sungkai leaves treat bruises, colds, fever, worms, and mouthwash to prevent dental disease\(^{(8)}\). Besides that, it is also used as a medicine for external wounds, internal wounds, anti-plasmodium, and dengue fever\(^{(8,9)}\). Based on the initial survey of the public from various backgrounds and professions that have been conducted, with a total of 21 respondents consisting of 13 people (18-23 years), one person (24-29 years), one person (30-35 years), one person (36-41 years), and five people (42-47 years), as much as 71% said that consuming Sungkai leaf decoction could make the body more fit, increase the body’s immunity, and relieve fever. The content of secondary metabolites in Sungkai leaves, such as flavonoids, alkaloids, tannins, and phenolics, is thought to have a high potential for anti-inflammatory activity\(^{(6,10)}\).

Anti-inflammatory activity can be determined by conducting in vitro and in vivo studies. The in vitro method has the advantage that the test time is faster, the sample is small, and it does not require test animals\(^{(11)}\). Research on anti-inflammatory activity in vitro can be done by inhibiting protein denaturation using bovine serum albumin\(^{(12)}\). Calculating the inhibitory concentration value can determine the effectiveness of inhibiting protein denaturation. If a compound can inhibit albumin denaturation by >20%, then it can be considered to have anti-inflammatory properties\(^{(6,11)}\). This study aimed to determine the highest flavonoid compound content in extracts and fractions to produce the best anti-inflammatory from Sungkai leaves.

MATERIAL AND METHODS

MATERIALS. Sungkai leaves (Peronema canescens Jack) were collected from the Pangkalan Balai city of Banyuasin South Sumatra Province in August 2022. Bovine serum albumin, 96% ethanol, distilled water, n-hexane, glacial acetic acid, NaCl, chloroform, HCl 2N, ethyl acetate, quercetin were purchased from Sigma-Aldrich, St. Louis, United States. 10% AlCl\(_3\) solution, 1M CH\(_3\)COONa solution, H\(_2\)SO\(_4\), FeCl\(_3\), Mg powder, Lieberman-Burchard reagent, Mayer reagent, Dragendorff’s, Wagner were purchased from Merck, tris base (Biogear), diclofenac sodium (Generic).

Equipments. Analytical balance (Mettler Toledo), pH metre (Mettler Toledo), water bath (Memmert), incubator (Memmert), oven (Memmert), vacuum rotary evaporator (Buchi). The instruments used in this study were UV-visible (Shimadzu UV-1900 Series, Kyoto, Japan) and FTIR spectrophotometers (Shimadzu, Kyoto, Japan).

METHODS. Extraction and Fractionation. Sungkai leaves (P. canescens Jack) were collected from the Pangkalan Balai city, washed with water, and dried and ground into powder form. Sungkai leaf powder (20 g) was put into three different containers. Add one litre of solvent (96% ethanol, ethyl acetate, and n-hexane) to each container. Then, the maceration results were filtered to obtain the filtrate. The filtrate obtained was evaporated using a rotary vacuum evaporator at 40°C to get viscous extracts of ethanol, ethyl acetate, and n-hexane, which were then tested for phytochemistry, and the yields of each were calculated.

The thick extract with the highest yield (ethanol) was then separated by fractionation using a separatory funnel with a ratio of ethanol to n-hexane (1:1) to obtain the ethanol fraction and n-hexane fraction.
Following their separation, the viscous ethanol extract, ethanol fraction, and n-hexane fraction underwent phytochemical screening as well as concentration changes to assess their anti-inflammatory effectiveness against protein denaturation inhibition\(^{(13,6)}\).

**Extract Yield Calculation.** The following formula can be used to determine the % yield of extracts with different solvents (ethanol, ethyl acetate, and n-hexane) and the results of ethanol and n-hexane fractionation can be calculated using the following formula\(^{(14)}\).

\[
\text{% Yield} = \frac{\text{Extract weight obtained}}{\text{The weight of the raw simplicia}} \times 100\%
\]

**Phytochemical Test.** The phytochemical tests carried out included alkaloid tests, flavonoid tests, tannin tests, saponin tests, terpenoid tests, and steroid tests\(^{(5,15)}\).

**Determination of Flavonoid Content.** After preparing a standard quercetin solution at a concentration of 1000 µg/ml, the maximum wavelength in the 400–800 nm range was determined with a UV–Vis spectrophotometer\(^{(10)}\). After adding 0.1 mL \(\text{AlCl}_3\), 0.1 mL \(\text{CH}_3\text{COONa}\) 1 M, and 2.8 mL distilled water to quercetin standard solutions at concentrations of 20, 30, 40, 50, and 60 ppm until homogenous, the mixture was left for 30 minutes. At a wavelength of 400–800 nm, the absorbance of each standard solution concentration was measured with a UV–Vis spectrophotometer. A UV–Vis spectrophotometer was used to measure the absorbance of the viscous extract, which was prepared at a concentration of 1000 ppm. The flavonoid content was then determined by calculating the absorbance value obtained using the quercetin standard curve\(^{(17,18)}\).

**Thin Layer Chromatography (TLC).** The eluent path was 8 cm since the TLC plate was created with a lower limit of 1 cm and an upper limit of 1 cm. Using a capillary tube, the ethanol extract, ethanol fraction, and n-hexane fraction were spotted at the plate’s lower edge. The acetone : ethanol (6:4) mobile phase was then used to elute the samples. Following the elution procedure, measure the retention factor value for each colour fluorescence and use a 254 nm and 366 nm UV lamp to observe the stain’s form\(^{(10)}\).

**Anti-inflammatory Activity Test In Vitro.** The ethanol extract, ethanol fraction, and n-hexane fraction of Sungkai leaves were tested for their anti-inflammatory properties in vitro, starting with the creation of TBS (Tris Buffer Saline). 400 millilitres of distilled water were mixed with 0.605 grammes of tris base and 4.35 grammes of NaCl. Glacial acetic acid should be used to bring the pH down to 6.2–6.5 before adding 500 mL of distilled water\(^{(6)}\). She poured 0.2 grammes of bovine serum albumin (BSA) into a 100 mL volumetric flask to create a solution. The TBS solution was then added to a 100 ml amount. The ethanol extract, ethanol fraction, and n-hexane fraction were dissolved in ethanol solvent up to the limit mark in a 25 mL volumetric flask to yield a concentration of 2500 ppm as the mother liquor. This process was repeated to create the test solution with different quantities of ethanol extract, ethanol fraction, and n-hexane fraction. Mother liquor was made into a test solution with concentrations of 1500, 1000, and 500 ppm by varying its concentration\(^{(6)}\).

The 25 mL measuring flask containing 62.5 mg of diclofenac sodium dissolved in distilled water was used as the positive control. Distilled water was added up to the tara mark to get a concentration that was comparable to the mother liquor. The concentration of the positive control solution was then adjusted in the mother liquor obtained, using values of 1500, 1000, and 500 ppm. In a measuring flask, 50 µL of distilled water and 0.2% BSA solution were dissolved until the volume reached 5 ml, which served as the negative control. Additionally, a test solution with concentrations of 5, 10, and 15 ppm was created. In order to determine the percent inhibition—percentage using linear regression and IC\(_{50}\) score conference—they measure the absorbance using UV-Vis. Using the following formula, the rate of protein denaturation inhibition was determined\(^{(6,20)}\).

\[
\% \text{Inhibition} = \frac{\text{Negative control absorbance} - \text{Absorbance of the test solution}}{\text{Negative control absorbance}} \times 100\%
\]

Over 20% inhibition of protein denaturation by compounds has anti-inflammatory effects and can serve as a development reference point. A linear regression equation between concentration and percentage inhibition was used to determine the IC\(_{50}\) value.

**RESULTS AND DISCUSSION**

**Yield of Extracts and Fractions.** Three different polarity solvents, like the non-polar n-hexane, the semi-polar ethyl acetate, and the polar ethanol, were used in the extraction process to separate the secondary metabolites according to their polarity (Table 1).

The results of the maceration of the ethanol extract produce a greenish-black colour. In contrast, the ethyl acetate extract is brownish black, and the n-hexane extract is greenish yellow. The yield of ethanol extract was the highest of ethyl acetate and n-hexane Extract, so it can be concluded that many of the secondary metabolite compounds of Sungkai leaves are polar. The highest yield of ethanol extract in Table 1 was
followed by fractionation with a separating funnel to remove non-polar metabolites using an $n$-hexane solvent. The weight of the dense section was taken 5 g to continue the fractionation process, whose yield value is displayed in Table 2.

The yield of the ethanol fraction obtained was (93.822%) This was thick and rather lumpy and had a dark brown colour, and the $n$-hexane fraction (4.81%) had a brownish-green colour with a smooth and thick consistency. Each sample’s unique texture is assumed to be influenced by the kind of secondary metabolite chemical that is attracted and the yield that is produced.

**Phytochemical Test.** Phytochemical tests were conducted to see qualitative measurement data on the condensed extract of Sungkai leaves (*P. canescens* Jack) from various solvents. The results of the phytochemical tests on the Sungkai leaf extracts in ethanol, $n$-hexane, and ethyl acetate are shown in Table 3. Based on Table 3, alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids are all present in the ethyl acetate extract. On the other hand, the ethanol extract has alkaloids, flavonoids, saponins, steroids, and tannins, whereas the $n$-hexane extract only has alkaloids, saponins, and steroids. The chemicals that are saponin, terpenoid, and steroid are the only differences between the fraction and the ethanol extract. Saponins are secondary metabolites that are alkaline and have a significant molecular weight. Hydrolyzed, it will produce an aglycone consisting of triterpenoid saponins and steroid saponins$^{(21,22)}$, while steroid compounds are hormones in plants usually derived from the terpenoid formation pathway$^{(23)}$. It is suspected that during the separation from the ethanol extract to the ethanol fraction, a reaction changed the initial structure of the saponins and steroids. The results were negative for these metabolites during the phytochemical identification.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extract Weight (g)</th>
<th>Fraction weight (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol Extract</td>
<td>11.28 g</td>
<td>4.6911 g</td>
<td>93.822</td>
</tr>
<tr>
<td>Ethyl Acetate Extract</td>
<td>3.82 g</td>
<td>0.2405 g</td>
<td>4.810</td>
</tr>
<tr>
<td>$n$-Hexane Extract</td>
<td>1.11 g</td>
<td></td>
<td>1.11 %</td>
</tr>
</tbody>
</table>

**Table 3. Phytochemical test results extracts and fraction of Sungkai leaves.**

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>Standard Test</th>
<th>Extracts</th>
<th>Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>$n$-Hexane</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>The red or orange precipitate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Yellow, orange, red, or green</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>There is foam</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>Purple or red color</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>Brown, green, or blue rings</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>Red precipitate</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: (+) There is a colour change according to the test; (-) No colour change
TLC (Thin Layer Chromatography). The results of the TLC analysis will be followed by a review of literature studies on isolating compounds found in Sungkai leaves. The following are the results of the TLC testing, which are presented in Table 4 and Figure 1.

The choice of eluent is based on the ability of the eluent to elute compounds. The eluent used in this TLC test was acetone: ethanol (6:4), based on research by Ardhica (19), which used the same plant extracts and eluent. According to Ardhica, the Sungkai leaf’s pure ethanol extract contained flavonoid compounds with an RF value of 0.71 (19). The results of the TLC test in Table 4 show the similarity of the RF values with Ardhica’s research, where the RF values of the ethanol extract and ethanol fraction were 0.75 and 0.72 and gave a blue glow in the 366 nm UV lamp; however, the colour fluorescence was still not wholly separated. However, it is suspected to have the same compounds as Ardhica’s research.

RF value of 0.76, the blue glow seen from UV 366 light is a secondary metabolite compound belonging to the flavonoid group. RF values can be used as evidence in identifying compounds. If the identification of the RF value has the same value, then the compound can have the same or similar characteristics. Based on a literature review, Ardhica has succeeded in isolating flavonoid compounds from the ethanol extract of Sungkai leaves, where the result is that at an RF value of 0.71 there is a flavonoid compound of the apigenin type (4’, 5, 7-trihydroxyflavone) with the molecular formula $C_{15}H_{10}O_5$.

<table>
<thead>
<tr>
<th>Eluent</th>
<th>Ethanol Extract (EE)</th>
<th>Ethanol Fraction (FE)</th>
<th>Fraction $n$-Hexane (FN)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone: Ethanol (6:4)</td>
<td>0.87</td>
<td>0.87</td>
<td>0.91</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>0.72</td>
<td>-</td>
<td>Blue</td>
</tr>
<tr>
<td></td>
<td>0.56</td>
<td>0.50</td>
<td>-</td>
<td>Fade Green</td>
</tr>
<tr>
<td></td>
<td>0.28</td>
<td>0.25</td>
<td>-</td>
<td>Turquoise</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.10</td>
<td>-</td>
<td>Dark green</td>
</tr>
</tbody>
</table>

Table 4. TLC results of ethanol extract, ethanol fractions, and fractions $n$-hexane.

To confirm the presence of apigenin in the ethanol extract, ethanol fraction, and $n$-hexane fraction, analysis was performed using FTIR (Fourier Transform Infra-Red). The FTIR spectrum of the ethanol extract, ethanol fraction, and $n$-hexane fraction can be seen in Figure 3.

The IR spectra of the ethanol extract and the ethanol fraction in Figure 3 have a similar absorption wave comparison to that of the apigenin compound in Ardhica’s study (19). Table 5 displays the functional groups and wave numbers’ comprehensive FTIR data.

According to Table 5, absorption in the OH groups ($3299 \text{ cm}^{-1}$ and $3326 \text{ cm}^{-1}$), C=O ketones ($1596 \text{ cm}^{-1}$ and $1596 \text{ cm}^{-1}$), and the ring C=C ($1445 \text{ cm}^{-1}$ and $1445 \text{ cm}^{-1}$) results in the ethanol extract and ethanol fraction containing apigenin-type flavonoid compounds and cyclic C-O ($1034 \text{ cm}^{-1}$ and $1034 \text{ cm}^{-1}$). The flavonoid compounds with anti-inflammatory activity are apigenin, baikalpin, fisetin, genistein, hesperidin, luteolin, and quercetin. From the TLC and FTIR data, it can be concluded that the extracts and fractions of Sungkai leaves from the Pangkalan Balai Province of South Sumatra also contain the same flavonoid compounds, namely Apigenin (4’,5,7-trihydroxyflavone).

**Determination of Flavonoid Levels.** According chromophores are atoms or groups contained in organic compounds that can absorb visible and
Table 6 shows that the n-hexane extract of Sungkai leaves (Peronema Cannes Jack) has more flavonoids than ethanol and ethyl acetate solvents. Flavonoids have a free form (aglycone) or are similar to glycosides, such as polyhydroxy aglycones, which are semi-polar, and polymethoxy aglycones, which are non-polar. In contrast, flavonoid glycosides are polar because they contain many hydroxyl groups and sugars. This means Sungkai leaves from Pangkalan Balai have many polar secondary metabolites. This compound is thought to be a group of flavonoids that have many hydroxyl (OH) groups. The hydroxyl group can act as a nucleophilic agent and be an active site in inhibiting inflammation for the body.

The quercetin calibration curve equation \( Y = 0.0115x + 0.018 \) is used to compare the concentration of flavonoid compounds in the sample. Flavonoids are secondary metabolites that are found in plants. These flavonoids were analysed using UV-Vis spectrophotometry because flavonoid compounds contain conjugated aromatic rings and can show strong absorption bands in the UV-Vis range. Table 6 shows the findings of measuring the amounts of flavonoids from the Sungkai leaf extracts in ethanol, n-hexane, and ethyl acetate.
Anti-Inflammatory Activity on Protein Denaturation Inhibition (in vitro). Testing the anti-inflammatory activity of protein denaturation is the simplest method to use. The protein used in this study is a protein from bovine serum albumin (BSA). Secondary metabolites of flavonoids found in the ethanol fraction and extract may have anti-inflammatory properties. Because flavonoids include hydroxyl groups and aromatic rings, they can interact with albumin amino acids to strengthen the structure of the protein\(^{(21)}\). The percentage inhibition of denatured protein can be used to calculate a compound’s anti-inflammatory effectiveness. A substance is said to have anti-inflammatory qualities if it can prevent albumin denaturation by more than 20%\(^{(5)}\).

Table 7 shows that the ethanol extract and fraction have an inhibition percentage value of >20%. A compound can inhibit albumin denaturation >20%; then, it can be considered as having anti-inflammatory properties\(^{(5)}\). Between the ethanol extract and the n-hexane fraction, the ethanol fraction exhibited the highest percentage of inhibition at concentrations of 5 ppm, 10 ppm, and 15 ppm. The ethanol fraction had more significant inhibition than the ethanol extract. However, during the phytochemical tests, the ethanol fraction did not contain secondary metabolites of the steroid and saponin groups. This shows that in this study, the secondary metabolites of the steroid group affect the anti-inflammatory activity to a lesser extent because steroids are not included in the non-steroidal anti-inflammatory drug (NSAID) class.

The inhibition of concentration is a parameter used to interpret the results of anti-inflammatory testing. The concentration of the test substance that reduces inflammation is known as the inhibition concentration value. Table 8 displays the concentration inhibition results for each sample.

Table 7. Percent inhibition of ethanol extract, ethanol fraction, n-hexane fraction and diclofenac sodium.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Inhibition (%) Ethanol Extract (A ± SD)</th>
<th>Ethanol Fraction (A ± SD)</th>
<th>Fraction n-Hexane (A ± SD)</th>
<th>Diclofenac Sodium (A ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>54.48 ± 0.0</td>
<td>74.27 ± 0.0</td>
<td>18.52 ± 0.0</td>
<td>70.34 ± 0.0</td>
</tr>
<tr>
<td>10</td>
<td>51.97 ± 0.0</td>
<td>70.19 ± 0.0</td>
<td>-7.68 ± 0.0</td>
<td>68.44 ± 0.0</td>
</tr>
<tr>
<td>5</td>
<td>50.23 ± 0.0</td>
<td>67.86 ± 0.0</td>
<td>-25.25 ± 0.0</td>
<td>65.78 ± 0.0</td>
</tr>
</tbody>
</table>

Table 8 Value of inhibition of concentration of Sungkai leaves and diclofenac sodium.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linear Regression</th>
<th>IC(_{50}) (µg/mL)</th>
<th>IC(_{70}) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol Extract</td>
<td>Y = 0.4255 X + 47.969</td>
<td>7.69 ± 0.0</td>
<td>51.77 ± 0.0</td>
</tr>
<tr>
<td>Ethanol Fraction</td>
<td>Y = 0.6414 X + 64.359</td>
<td>-20.60 ± 0.0</td>
<td>8.79 ± 0.0</td>
</tr>
<tr>
<td>n-Hexane Fraction</td>
<td>Y = 4.3771 X - 48.575</td>
<td>23.39 ± 0.0</td>
<td>27.08 ± 0.0</td>
</tr>
<tr>
<td>Diclofenac Sodium</td>
<td>Y = 0.4563 X + 63.625</td>
<td>-19.27 ± 0.0</td>
<td>13.97 ± 0.0</td>
</tr>
</tbody>
</table>

CONCLUSION

The highest levels of flavonoids were in the ethanol extract of Sungkai leaves (P. canescens Jack), equal to 38.782 µg/mL. The ethanol extract and fraction had the best anti-inflammatory activity against protein denaturation inhibition at 54.48% and 74.27%. The best IC\(_{50}\) and IC\(_{70}\) values were the ethanol fraction > n-hexane fraction > ethanol extract.

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