JURNAL ILMU KEFARMASIAN INDONESIA, October 2023, page. 223-231 Vol. 21, No. 2
ISSN 1693-1831

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)

DESI NADYA AULENA[1,2], DWI FITRI YANI[3*], MARIYAMAH[3], MUHAMMAD LUFIKA TONDI[3], MUHAMMAD DANDI[3], HAFIS KIKI WAHYUDIN[3], DANY RAIHAN[4]

[1]Departement of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Pancasila, South Jakarta, Jakarta, 12640, Indonesia
[2]Center for The Study of Natural Product for Degenerative Diseases, Faculty of Pharmacy, Universitas Pancasila, South Jakarta, Jakarta, 12640, Indonesia
[3]Departement of Chemistry, Faculty of Sains and Technology, Universitas Islam Negeri Raden Fatah Palembang, South of Sumatera, 30257, Indonesia
[4]Faculty of UBC Vantage College, University of British Columbia, Vancouver, V6T 1Z4, Canada

Submitted 11 July 2023, Accepted 29 September 2023

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 IC_{50} and IC_{70} 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 IC_{50} and IC_{70} 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 peradangan 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 menggunakan 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, masing-masing sebesar 74,27% dan 54,4 persen. Ekstrak etanol daun sungkai memiliki kadar flavonoid tertinggi sebesar 38.782 g/mL. Nilai IC_{50} dan IC_{70} 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.

*Corresponding author
e-mail: dwifitriyani_uin@radenfatah.ac.id

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%)[1]. 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[2]. 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 combat and control 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

MATERIASL. 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_3COONa solution, H_2SO_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].

$$\% \text{ Yield} = \frac{\text{Extract weight obtained}}{\text{The weight of the raw simplicia}} \text{ x } 100\%$$

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[16]. After adding 0.1 mL $AlCl_3$, 0.1 mL CH_3COONa 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[19].

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[5]. 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 tera 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].

$$\% \text{ Inhibition} = \frac{\text{Negative control absorbance} - \text{Absorbance of the test solution}}{\text{Negative control absorbance}} \text{ x } 100\%$$

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 1. Yield of extracts of Sungkai leaves (*P. Canescens* Jack).

Sample	The Weight of Simplisia	Extract Weight	Yield (%)	Yield Image
Ethanol Extract	100 g	11.28 g	11.28 %	
Ethyl Acetate Extract	100 g	3.82 g	3.82 %	
n-Hexane Extract	100 g	1.11 g	1.11 %	

Table 2. Yield of fraction Sungkai leaf (*P. canescens* Jack).

Sample	Extract Weight (g)	Fraction weight (g)	Yield (%)
Ethanol Fraction	5	4.6911	93.822
n-Hexane Fraction		0.2405	4.810

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

Phytochemical Test	Standard Test	Extracts			Fraction	
		Ethanol	*n*-Hexane	Ethyl Acetate	Ethanol	*n*-Hexane
Alkaloid	The red or orange precipitate	+	+	+	+	-
Flavonoid	Yellow, orange, red, or green	+	-	+	+	-
Saponin	There is foam	+	+	+	-	-
Terpenoid	Purple or red color	-	-	+	-	-
Steroids	Brown, green, or blue rings	+	+	+	-	+
Tannin	Red precipitate	+	-	+	+	-

Note: (+) There is a colour change according to the test; (-) No colour change

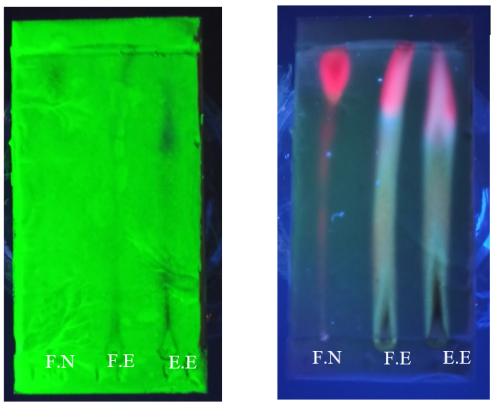
TLC (Thin Layer Chromatography). The results of the TLC analysis will be followed by a review of literature studies on isolating compounds found in Sungkai leaves. The following are the results of the TLC testing, which are presented in Table 4 and Figure 1.

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[24]. 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[10]. 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 trhydroxyflavone) with the molecular formula $C_{15}H_{10}O_{5.}$

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

Eluent	Retention Factor			Color
	Ethanol Extract (EE)	Ethanol Fraction (FE)	Fraction *n*-Hexane (FN)	
Acetone: Ethanol (6:4)	0.87	0.87	0.91	Red
	0.75	0.72	-	Blue
	0.56	0.50	-	Fade Green
	0.28	0.25	-	Turquoise
	0.15	0.10	-	Dark green



(a) (b)

Figure 1. (a) TLC results of 254 nm UV lamp, (b) TLC results of 366 nm UV lamp.

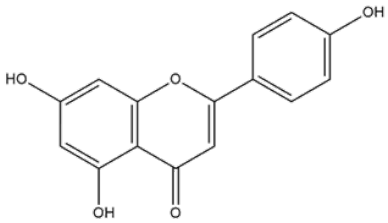


Figure 2. Apigenin (4', 5, 7-trhydroxyflavone).

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 cm^{-1} and 3326 cm^{-1}), C=O ketones (1596 cm^{-1} and 1596 cm^{-1}), and the ring C=C (1445 cm^{-1} and 1445 cm^{-1}) results in the ethanol extract and ethanol fraction containing apigenin-type flavonoid compounds and cyclic C-O (1034 cm^{-1} and 1034 cm^{-1})[19]. The flavonoid compounds with anti-inflammatory activity are apigenin, baikalin, fisetin, genistein, hesperidin, luteolin, and quercetin[1]. 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-trhydroxyflavone).

Determination of Flavonoid Levels. According chromophores are atoms or groups contained in organic compounds that can absorb visible and

ultraviolet light, such as alkenes, alkynes, carbonyls, carboxyls, amides, azos, nitro, nitroso, and nitrates[25]. Meanwhile, autochromes are functional groups with free electrons, such as -OH, -O, $-NH_2$, and $-OCH_3$. The results of absorbance measurements of the quercetin standard solution are shown in Figure 4.

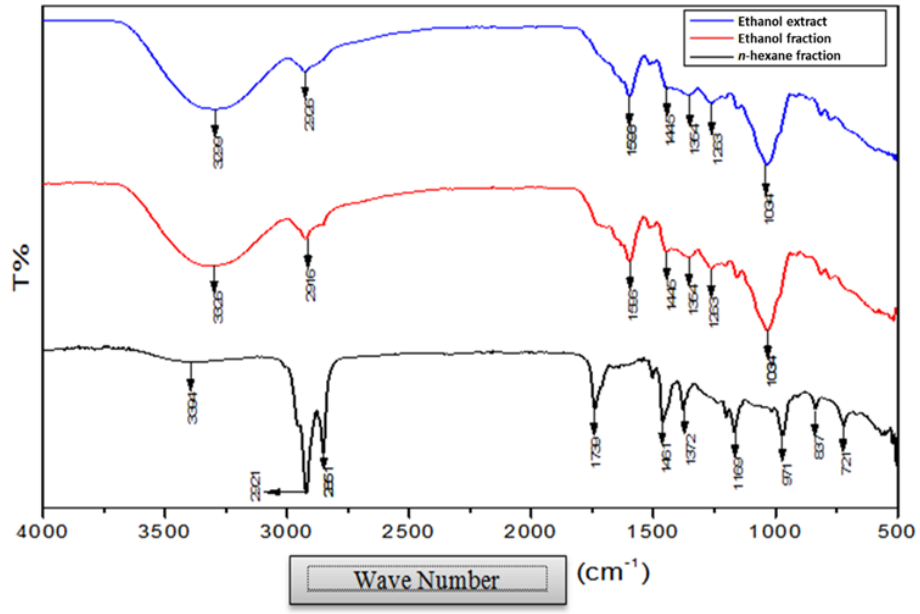


Figure 3. IR spectrum of ethanol extract, ethanol fraction, and *n*-hexane fraction.

Table 5. Identification of IR wave numbers[19].

Functional groups	Wave Number (cm^{-1})				
	Ethanol Extract	Ethanol Fraction	Fraction n-Hexane	Apigenins (Ardhica, 2022)	Apigenins (Shoubaky, 2016)
O-H *stretch*	3299	3326	-	3331	3333
C=O	1596	1596	1739	1607	1646
C=C	1445	1445	1461	1442	1466
C-O *stretch*	1034	1034	-	1029	1024

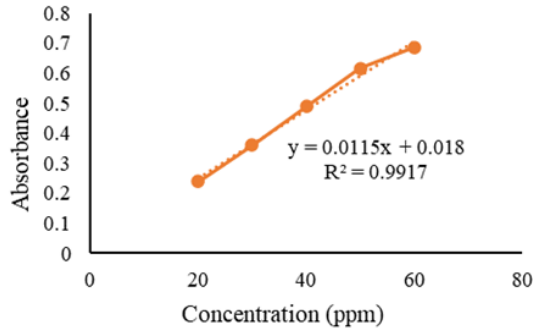


Figure 4. Quercetin calibration curve at λ_{maks} = 413 nm.

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[15]. Table 6 shows the findings of measuring the amounts of flavonoids from the Sungkai leaf extracts in ethanol, *n*-hexane, and ethyl acetate.

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[5,26]. 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.

Table 6. Results of flavonoid levels in Sungkai leaves (*P. Cannes* Jack).

Sample	Concentration (ppm)	Absorbance (A ± SD)	Flavonoid levels (µg/mL)
Ethanol extract	1000	0.464 ± 0.0	38.782
Ethyl acetate extract	1000	0.112 ± 0.0	8.173
n-hexane extract	1000	0.06 ± 0.0	3.652

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 8 shows the good IC_{50} and IC_{70} values of ethanol extract, ethanol fraction, n-hexane fraction, and diclofenac sodium ethanol fraction. The lower the IC_{70} value, the better the anti-inflammatory, so the ethanol fraction > diclofenac sodium > n-hexane fraction > ethanol extract was the best. However, for the n-hexane fraction, the inhibition percentage did not reach 20%, so it is suspected that the ethanol extract will provide better inflammatory activity than the n-hexane fraction[27].

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

Concentration (ppm)	Inhibition (%)			
	Ethanol Extract (A ± SD)	Ethanol Fraction (A ± SD)	Fraction *n*-Hexane (A ± SD)	Diclofenac Sodium (A ± SD)
15	54.48 ± 0.0	74.27 ± 0.0	18.52 ± 0.0	70.34 ± 0.0
10	51.97 ± 0.0	70.19 ± 0.0	-7.68 ± 0.0	68.44 ± 0.0
5	50.23 ± 0.0	67.86 ± 0.0	-25.25 ± 0.0	65.78 ± 0.0

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

Sample	Linear Regression	IC_{50} (µg/ mL)	IC_{70} (µg/mL)
Ethanol Extract	Y = 0.4255 X + 47.969	7.69 ± 0.0	51.77 ± 0.0
Ethanol Fraction	Y = 0.6414 X + 64.359	-20.60 ± 0.0	8.79 ± 0.0
n-Hexane Fraction	Y = 4.3771 X – 48.575	23.39 ± 0.0	27.08 ± 0.0
Diclofenac Sodium	Y = 0.4563 X + 63.625	-19.27 ± 0.0	13.97 ± 0.0

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.

ACKNOWLEDGMENTS

The authors would like to thank the Faculty of Sains and Technology, Universitas Islam Negeri Raden Fatah Palembang and Faculty of Pharmacy, Universitas Pancasila, Indonesia, for allowing this study to be conducted in their laboratory.

FUNDING

This research did not receive any specific grants from public, commercial, or non-profit funding agencies.

REFERENCES

1. Hairunisa N, Amalia H. A review of coronavirus disease-2019 (COVID-19). Jurnal Biomedika dan Kesehatan. 2020;3(2):90–100.
2. Wahyuni IS, Sufiawati I, Nittayananta W, Levita J. Anti-inflammatory activity and wound healing effect of *Kaempferia galanga L*. Rhizome on the chemical-induced oral mucosal ulcer in wistar rats. Journal of Inflammation Research. 2022;15:2281-94.
3. Robert B, Brown EB. Pathophysiology: Clinical concepts of disease processes. EGC Medical Book Publishers. 2004;(1):1–14.
4. Novika DS, Ahsanunnisa R, Yani DF. Anti-inflammatory activity of ethanol extract of starfruit leaves (*Averrhoa bilimbi L*.) against inhibition of protein denaturation. 2021;3(1):16–22.
5. Reynaldi, Yani DF. The Anti-inflammatory potential of cocor bebek leaves (*Kalanchoe pinnata* L.) against in vitro protein denaturation. Jurnal Kimia dan Pendidikan Kimia. 2021;3(1):12–21.
6. Fatahillah R, Fitriyani D, Wijayanti F. In vitro anti-inflammatory activity of extract and fraction seed coat kebiul (*Caesalpinia bonduc* L.). Al-Kimia. 2022;10(1):42–50.
7. Jang M, Jeong SW, Cho SK, Ahn KS, Lee JH, Yang DC, Kim JC. Anti-inflammatory effects of an ethanolic extract of guava (*Psidium guajava L.*) leaves in vitro and in vivo. J Med Food. 2014 Jun;17(6):678-85.
8. Sitepu NB. In Vitro Test of Antibacterial ethanol extract, n-hexane fraction and ethyl acetate fraction of Sungkai leaf (*Peronema cenescens*) against *Salmonella typhi*. Asian Journal of Pharmaceutical Research and Development. 2020;8(3): 57-60.
9. Prasiwi D, Sundaryono A, Handayani A. The activity of the *Peronena canescens* leaves ethanol extract fraction against *Plasmodium berghei* growth rate. Jurnal Pendidikan dan Ilmu Kimia. 2018;2(1):25-32.
10. Masyita M, Sayekti E, Nurlina N. Flavonoid compounds of the catechin from wungu (*Graptophyllum pictum* (L.) Griff) leaves and the sun protecting factor value. Jurnal Akademi Kimia. 2022;11(1):31–8.
11. Kumari CS, Yasmin N, Hussain MR, Babuselvam M. In vitro anti-inflammatory and anti-arthritic property of *Rhizopora Mucronata* leaves. International Journal of Pharma Sciences and Research (IJPSR). 2015;6(3):482-85.
12. Farida Y, Rahmat D, Widia Amanda A. Anti-inflammation activity test of nanoparticles ethanol extract of temulawak rhizome (*Curcuma xanthorrhiz*a). Jurnal Ilmu Kefarmasian Indonesia. 1264;16(2):225–30.
13. Yani DF, Sugita P, Syahbirin G. Phytochemicals and cytotoxicity of sausage fruit (*Kigelia africana*) extract against breast cancer cells MCF-7 in vitro. J Pharm Res. 2018;12(3):288–92.
14. Fitri Yani D, Dirmansyah R. Activity test of methanol and n-hexan fraction of coat and kernel seed (*Caesalpinia bonduc l.*) as a sun screen. Jurnal Sains Dasar. 2021;10(1):1–5.
15. Banu KS, Cathrine. General technique involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science (IJARCS). 2015;2(4):25-32.
16. Ahmad AR, Saleh RH, Handayani V. Standardization and characterization of essential oil of patchouli stem (*Pogostemon Cablin Benth.*) by chromatography-mass spectrometry (GC-MS) method. Journal of Pharmaceutical Negative Results. 2022; 13:4218-31.
17. Nurcholis W, Putri DNSB, Husnawati H, Aisyah SI, Priosoeryanto BP. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits. Annals of Agricultural Sciences. 2021;66(1):58-62.
18. Desmiaty Y, Elya B, Saputri FC, Dewi I, Hanafi M. Effect of extraction method on polyphenol content and antioxidant activity of *Rubus fraxinifolius*. Jurnal Ilmu Kefarmasian Indonesia. 2019;17(2):227-31.
19. Tarigan IL, Aini IPS, Latief M. Isolation of a flavone apigenin and a steroids squalene from *Peronema canescens Jack* leaves with anti-inflammatory activities. Pharmacognosy Journal. 2022;14(6):744-52.
20. Williams LAD, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, et al. The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat-treated (immunogenic) bovine serum albumin is proposed as a screening assay for detecting anti-inflammatory compounds without using animals. West Indian Med Journal. 2008;57(4):327–31.
21. Bailey-shaw Y a, Williams L a D, Green CE, Rodney S, Smith AM. In-vitro evaluation of the anti-inflammatory potential of selected Jamaican plant extracts using the bovine serum albumin protein denaturation assay. International Journal of Pharmacy Science Rev Res. 2017;47(1):145–53.
22. Saleem A, Saleem M, Akhtar MF. The antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: An ethnomedicinal plant of moringaceae family. South African J Bot. 2020;128:246–56.
23. Sari IP, Hariyanti H, Yanuar A, Hayun H. New decahydroacridine-1, 8-diones derived from 3-aminocyclohex-2-en-1-one: synthesis, characterization, antioxidant, in-vitro, and in-silico anti-inflammatory activity. Rasayan Journal of Chemistry. 2022;*15*(2):1241-48.

24. Koirewoa YA, Fatimawali, Wiyono WI. Isolation and identification flavonoid compounds in Beluntas leaf (*Pluchea Indica* L .). Jurnal Farmasi. 2012;47–52.
25. Latief M, Fisesa AT, Sari PM, Tarigan IL. Anti-inflammatory activity of Sungkai leaves (*Peronema Canescens* Jack.) ethanol extract in carrageenan induced mice. Jurnal Farmasi Sains dan Praktek. 2021;7(2):144–53.
26. Ghumre SV. Assessment of in-vitro anti-inflammatory activity of cynodon dactylon and acyclovir showing synergistic effect by albumin denaturation and membrane stabilization assay. Modul Approaches Drug Des. 2017;1(2):1–5.
27. Ali AM, El-Nour M, Mohammad O, Yagi SM. In vitro, anti-inflammatory activity of ginger (*Zingiber officinale Rosc.)* rhizome, callus and callus treated with some elicitors. Journal of Medicinal Plants Research. 2019;*13*(10):227-35.