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Original Article

Comparison of the Anti-Inflammatory Activity of Flavonoid Bioactive Compounds Acetone Fraction and Steroid Fraction Ethyl Acetate Sungkai Leaves *In Vivo* and In Silico Studies

Madyawati Latief[1,2], Rahmani[1], Arliangga Fahrezi[1], Sutrisno[1], Indra Lasmana Tarigan[1,2*]

ABSTRACT

One of the medicinal plants that is widely used by traditional communities is sungkai (*Peronema canescens* Jack), which is known to contain secondary metabolite compounds of flavonoids, saponins, tannins, steroids, terpenoids, alkaloids, and phenols. This research aims to isolate secondary metabolite compounds from acetone and ethyl acetate fractions, which act as anti-inflammatory agents in vivo and in silico. Acetone and ethyl acetate fractions were isolated and characterized using UV-Vis and FT-IR. The In Vivo test was carried out on Mus musculus, while the In Silico test was carried out using the IL-10 and IFN-γ receptors, which play a role in inflammation and immunity. Our research results showed that the ethanolic (F1) and n-hexane (F2) fractions of *P. canescens* extract had good anti-inflammatory activity with percent inhibition values of 58.12% and 56.59%, respectively. The characterization results showed that isolate F1 was a flavonoid group, Naringenin compound, while isolate F2 was a steroid group, β-Sitosterol compound. Moreover, from the scoring docking results, β-Sitesterol has tremendous potential as an anti-inflammatory than the compound naringenin.

Key words: Anti-inflamatory; β-Sitesterol; Naringenin, *P. canescens* Jack.

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INTRODUCTION

Peronema canescens Jack is a medicinal plant,[1] traditionally used to treat fever.[2] The phytochemical screening test of the ethanol extract of sungkai leaves contained flavonoids, phenols, tannins, alkaloids and saponins.[3] The methanol extract of sungkai leaves contains secondary metabolite compounds, namely alkaloids, flavonoids, terpenoids and steroid.[3] Bioactivity assys is carried out to determine the biological activity of the test sample, including anti-inflammatory testing. Test samples consist of fractions, crude extracts and pure compounds from natural ingredients.[4] Inflammation is the body's attempt to activate or damage organisms that attack the body, eliminate irritants and increase the degree of tissue repair.[5] When inflammation occurs in the body, the treatment can be done by consuming steroids and non-steroidal drugs. Steroid drugs work by inhibiting the formation of arachidonic acid, while non-steroidal drugs work by inhibiting the formation of prostaglandins.[6] Sungkai leaves are an alternative that can be used to find a more natural source of anti-inflammatory compounds.[7] The ethanol extract of sungkai (*Peronema canescens* Jack) has a wide range of activities. In previous research, it was found that the ethanol extract of sungkai leaves had anti hyperuricemia activity, namely by reducing uric acid levels in the blood of mice.[8] At a dose of 0.056 g/Kg BW, the ethyl acetate fraction can inhibit parasitemia growth by 50.89%, a more significant percentage of inhibition than negative and positive controls.[9] The compounds in sungkai leaf extract act as anti-inflammatory candidates, namely flavonoids, saponins, alkaloids and phenols. Anti-inflammatory compounds play a role in inhibiting the formation of prostaglandin mediators, inhibiting the migration of leukocyte cells to the area of inflammation and inhibiting the release of prostaglandins in the cells. Then, the ethanol extract of sungkai leaves has an anti-inflammatory level. Sungkai leaf extract with a concentration of 15% cannot yet approach the anti-inflammatory effect of hydrocortisone acetate 2 .5% with an inhibition percentage reaching >50%.[5] Our previous research has isolated the flavone compound Apigenin and the steroid Squalene from the ethanol and n-hexane extracts of Sungkai leaves. The two isolated compounds have good anti-inflammatory activity with inhibition values of 58.12% and 56.59%, respectively.[7] This research was conducted to isolate the acetone and ethyl acetate fractions of Sungkai leaves as a follow-up to previous results and to analyze differences in anti-inflammatory activity in vivo against *Mus musculus* induced by carrageenan and molecular docking analysis using IL-10 receptors and IFN-γ which plays a role in inflammation and immunity.

MATERIAL AND METHODS

Chemicals and Equipments

The samples used were Sungkai leaves obtained from Kademangan Village, Jaluko District, Muaro Jambi Regency, Jambi Province, Indonesia. The Sungkai plant was identified by the Biotechnology, Agroindustry and Medicinal Plants Laboratory, Faculty of Science and Technology, Jambi University. They were identified by the Biotechnology and Agroindustry Laboratory of Medicinal Plants, Faculty of Science and Technology, Jambi University. Chemicals used are Acetone, Sulfuric Acid, Ethyl

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Acetate, Phytochemical Reagents, Silica Gel, HCl, $FeCl_3$, Anhydrous acetic acid, Na-CMC, Na-Dichlorofenac, and Carrageenan (Sigma-Aldrich). The animal used was *Mus musculus*, aged 2 – 3 months, weighing 15 – 30 g, which had been acclimatized to adapt to environmental conditions for one week and had received ethical permission from the Faculty of Medicine and Health Sciences, Jambi University. The instrumentation used is glassware, a Rotary Evaporator (Buchi R-300), a Pletysmometer, a UV-Vis Spectrophotometer, and FT-IR (Thermo-Scientific).

Phytochemical Extraction and Screening

Samples were collected, cleaned, washed, dried naturally, and ground to obtain simplicia. Next, it is extracted in stages using non-polar solvents up to polar solvents. This research explicitly used the results of graded fractions of acetone and ethyl acetate. The following fraction was concentrated using a Rotary Evaporator. Phytochemical screening was performed on the viscous fraction by analyzing phenolic compounds, steroids, flavonoids, alkaloids and saponins.[10]

Purification and Isolation

Thin layer chromatography (TLC) was used to identify fraction stain patterns using a 1 x 5 cm TLC plate. An eluent with a gradient level of polarity is used. The stain patterns were analyzed using a UV lamp with a wavelength of 254 nm, and similar stain patterns were then grouped. Isolation was carried out using Column Chromatography using silica gel as the stationary phase with a ratio of sample: silica gel (1:20). The fraction is impregnated, and a mobile phase (solvent) with a polarity gradient is used. Column chromatography results were carried out using TLC again. The Rf value in the chromatogram if there are identical stains.[11]

Compound Characterization

Characterization of the isolated compounds was carried out using several instrumentations in stages. UV-Vis Spectrophotometer: 2 mL of the isolate was placed in a cuvette, and the spectrum was analyzed at 200 – 800 nm wavelength to obtain the maximum wavelength. FT-IR Spectrophotometer: 0.2 g of KBR plate was added with one drop of isolate, dried, and identified using an FT-IR spectrophotometer at wave numbers 400 – 4000 $cm^{-1.}$

Anti-Inflammatory Activities

The activity of anti-inflammatory compounds was analyzed using male white mice with criteria of body weight 20-30g, 2-3 months old, and in healthy and normal condition. Before testing the experimental animals were adapted to the environment for ± one week. All test animals were kept under the same conditions. The cages were conditioned at room temperature and there was a light and dark cycle every 12 hr. Before giving the treatment, they were given enough food and drink. During the experiment, food, and drink were placed ad libitum.[5]

The anti-inflammatory activity was carried out using a combination method of forming air sacs and artificial oedema on the backs of mice with a subcutaneous carrageenan solution.[12] Anti-inflammatory activity tests were conducted on positive control (Na-diclofenac), negative control (Na CMC), sungkai leaf acetone extract, fractions and isolates. Before treatment, mice were fasted for 18 hr (not given food but given water). Each consists of 3 mice. The negative controls used were Na-CMC (1%), and the Positive Control was Na-dichlorofenac. The acetone and ethyl acetate fraction used is 300mg/KgBW, 600mg/KgBW, 900mg/KgBW. Meanwhile, the anti-inflammatory activity of the isolate used at 10 mg/kgBW for isolates was evaluated with three variations of 3, 5 and 10 mg/kgBW.

Molecular Docking

Macromolecular Preparation

The three-dimensional macromolecules IL-10 and IFN-γ were downloaded from the Protein Data Bank data site https://www.rcsb.org. The PDB codes used are 1LQS (IL-10) and 3BES (IFN-γ). Macromolecules are separated from solvents and native ligands or non-standard residues using the UCSF Chimera application. *Validation of Molecular Docking Parameters:* Validation of the molecular docking method was carried out using AutoDock Tools software. This is done by re-docking the natural ligands of each macromolecule (receptor). The parameter used is Root Mean Square Deviation (RMSD). The results obtained in this process are the grid box parameters and RMSD values. The docking method is said to be valid if it has an RMSD value < 3 Å, this value indicates the protocol is accepted and docking can be carried out. *Molecular Docking:* The molecular docking process is carried out using PyRx software based on AutoDock Tools. The macromolecule (receptor) and ligand structures that have been optimized separately are stored in one folder. The molecular docking process uses a grid box and energy minimization parameters according to validation results. Grid box parameter settings are carried out using grid box coordinates which are determined based on the ligand coordinates of the receptor used in the docking validation process. Next, docking is carried out using PyRx software with the AutoDock wizard feature. The docking data displayed is in the form of binding affinity values and amino acid residue interactions. Docking results are saved in pdb format.

Data Analysis

Anti-inflammatory activity was determined by calculating the inflammation volume and percent inhibition of the sample. The data was then tested with One-Way ANOVA and Post Hoc LSD test with a confidence level of 95% using SPSS 25 software.[13]

RESULTS AND DISCUSSION

% Yield and Phytochemical Screening

The yield value is calculated from the multilevel extraction results to determine the ratio of the weight of the dry material produced to the weight of the initial raw material. The yield results of the three sungkai leaf fractions can be seen in Table 1.

Based on Table 1, the ethyl acetate and ethanol fractions yield value is higher than the other fractions because most of the compounds contained in sungkai leaves have almost the same polarity as certain solvents, so these compounds tend to dissolve in these solvents. Previous studies reported that, ethyl acetate is a semipolar compound capable of binding polar and nonpolar compounds [9]. Ethanol is a universal solvent because it can attract polar, semipolar and nonpolar compounds. After all, a hydroxyl group (-OH) can bond with polar compounds, and the ethyl group can attract all nonpolar and semipolar components.[14] Two fractions of n-hexane and EtOH have been reported in previous research, so this research was carried out on two other fractions, Ethyl acetate and Acetone.

In the process of separating the acetone fraction, the result was that it contained 22 vials and then evaporated at room temperature until approximately ½ of the vials. After that, TLC was carried out on each vial to identify the fractions in the 22 vials. Vials that have the same stain pattern on TLC are combined into one fraction; the results of this Vacuum Liquid Chromatography (VLC) are three fractions, which are symbolized by fraction 1 (F1), fraction 2 (F2) and fraction 3 (F3).

In the ethyl acetate fraction, vials with the same stain pattern were combined, and five combined fractions were obtained, coded F1, F2,

Table 1. **% Yield of the Sungkai Leaves fraction.**

Fraction	% Yield	References
n-Hexane	12.0	7
Ethyl Acetate	18.99	This Studies
Acetone	12.8	This Studies
EtOH	28.70	7

Table 2. **Phytochemical screening of Acetone fractions.**

Compounds	Acetone	F1	F2	F3	Isolate
Alkaloids	-	-	-	-	-
Flavonoids	+	+	+	+	+
Phenolics	+	+	+	+	-
Saponins	-	-	-	-	-
Steroids	+	-	-	-	-
Terpenoids	-	-	-	-	-

Table 3. **Phytochemical screening of Ethyl acetate (EtAce) fractions.**

Compounds	EtAce	F1	F2	F3	F4	F5	Isolate
Alkaloids	+	+	+	+	+	+	
Flavonoids	+						
Phenolics	+						
Saponins	-						
Steroids	+	+	+	+	+	+	+
Terpenoids	-						

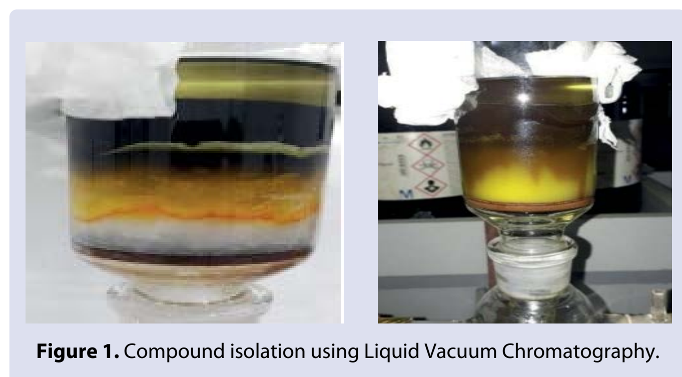

Figure 1. Compound isolation using Liquid Vacuum Chromatography.

F3, F4 and F5. In F1, the combined results were obtained for vials 1-3, F2 combined vials 4-8, F3 combined vials 9-11, F4 combined vials 12-14 and F5 combined vials 15-25. Based on the VLC results, there was a crystalline precipitate at the bottom of the vial in vials 6-8 with the eluent ratio n-hexane: ethyl acetate (6:4). Then the precipitate was recrystallized using n-hexane and ethyl acetate solvents to separate the impurities that came down during the elution process. After recrystallization, white crystal grains were obtained. Furthermore, the crystals in TLC with the eluent n-hexane: ethyl acetate (6:4) showed a single stain pattern.

Phytochemical screening was carried out qualitatively using phytochemical reagents for each metabolite compound. The extract was analyzed for its chemical compound content using a colour test using several reagents. This test is carried out to determine the presence of alkaloids, flavonoids, steroids or terpenoids, tannins, saponins and phenols.

The results of the phytochemical screening showed that the ethyl acetate and acetone fractions positively contained steroid and flavonoid metabolites. All fractions showed positive results when the Dragendorf test was carried out, while the Mayer reaction showed negative results.

Re-crystallization was carried out to purify the compound and analyzed using TLC with various solvents ethyl acetate: methanol (6:4) with an Rf value of 0.65, and a pure isolate of 0.54 g was obtained. Meanwhile, the ethyl acetate fraction produced a single stain as a result of TLC analysis using DCM solvent: n-hexane (6:4); DCM: Ethyl acetate (6:4); DCM (100%) and obtained pure isolate 0.48 g.

Characterization

Acetone isolate (AI) has been characterized using UV-Vis and FT-IR instruments. UV-Vis characterization was carried out to determine the essential compounds of the isolate. UV-Vis aims to determine the basic framework of the isolated compound, namely conjugated double bonds, types of chromophores and autochromes in organic compounds. The UV-Vis spectrum shows that the isolate gives two absorption peaks: band one λ = 356.91 nm and band two λ = 663.61 nm. Absorption at 356 nm indicates the presence of a conjugated diene/dual system in the structure.

Meanwhile, the absorption at 663 nm indicates the presence of an aromatic system with particular substituents. Based on literature comparisons, the UV-Vis spectrum of this particular isolate has a similar pattern to the UV-Vis spectrum of naringin in Figure 1-6. However, in band 2, the standard naringin is λ = 663.61 nm. There is a reasonably significant shift between isolate band two and band 2 of the naringin standard; this may be due to the influence of concentration during testing. Meanwhile, the results of the analysis of ethyl acetate isolates provide results in the form of a spectrum with maximum absorption bands at wavelengths of 269 nm and 335 nm, which are characteristic of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. The appearance of maximum absorption at a wavelength of 269 nm seems to result from 29 electron transitions from groups with lone pairs of electrons.

Meanwhile, the $\pi \rightarrow \pi^*$ electronic transition at a wavelength of 335 nm occurs from the group with a double bond from the ring of the steroid compound. FT-IR characterization aims to identify functional groups in a compound based on differences in absorption at wave numbers. The following is the IR spectrum of isolates from the acetone and ethyl acetate fractions of sungkai leaves in the picture and the comparison compounds.

Based on the IR spectrum of the F3 isolate from the sungkai leaves acetone fraction, it shows that there is stretching vibration absorption from O-H in the 3347.11 cm-1 area, absorption in the 2880.64 cm-1 area shows the presence of –CH aliphatic, stretching vibrations from –C=O in the absorption area of 1638.91 cm-1, stretching vibrations in the absorption area of 1489.44 cm-1 which indicate the characteristics of aromatic C=C, and vibrations in the absorption area of 1035.29 cm-1 which indicate the characteristics of carbonyl compounds. This IR absorption is significant in indicating the characteristics of the flavonoid secondary metabolite group of sub-flavonones. Based on a comparison of the IR spectrum shows that the compound is close to the IR spectrum of naringenin.

Meanwhile, Isolate F2 Ethyl acetate Fraction produced an absorption at a wave number of 3254.05 cm^{-1}, which indicates the presence of stretching absorption of the O-H group. Furthermore, at wave number 2994.62 cm^{-1} with extreme intensity, indicating the presence of aliphatic C-H stretching vibrations. Absorption in the wavelength range 1650-1500 cm^{-1}, namely at 1667.53 cm^{-1}, indicates the presence of the C=C group, then at the wave number 1434.48 cm^{-1} indicates the presence of the $-CH_2$ group, the sharp absorption band is between 1300-1000, namely at the wave number 1119.73 cm^{-1} indicates the absorption of the C-O group. This data shows that the isolated compound F2.8 contains hydroxyl groups (O-H), aliphatic CH, $-CH_2$, C=C and C-O Ether. The absorption band showing the presence of these groups gives an idea that the isolated compound may be a cyclic steroid compound containing an OH group.

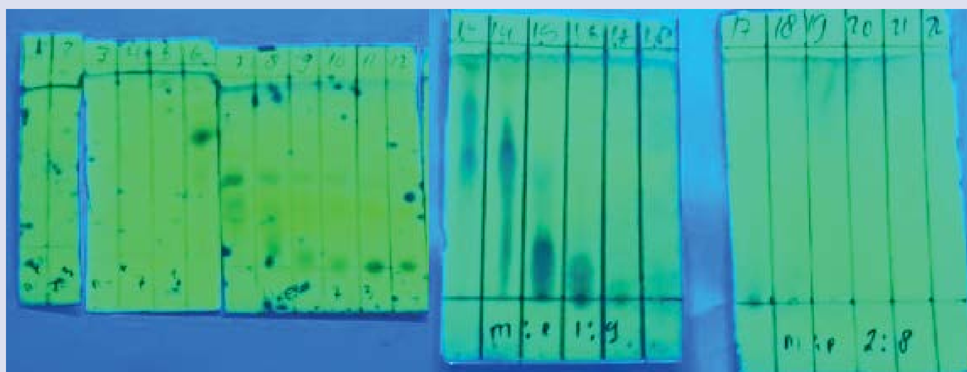

Figure 2. TLC results and incorporation of the acetone fraction resulting from VLC.

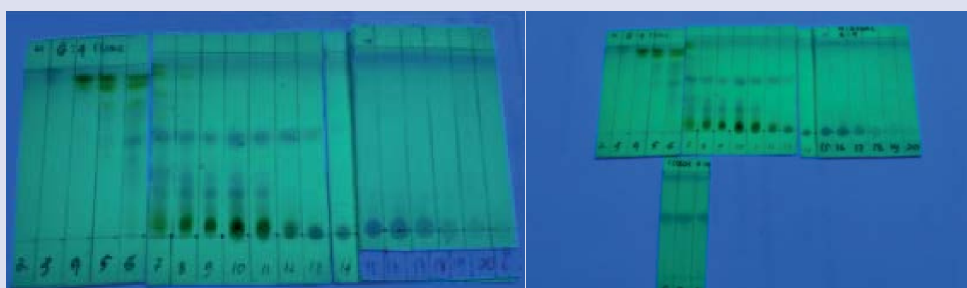

Figure 3. TLC results and incorporation of the ethyl acetate fraction resulting from VLC.

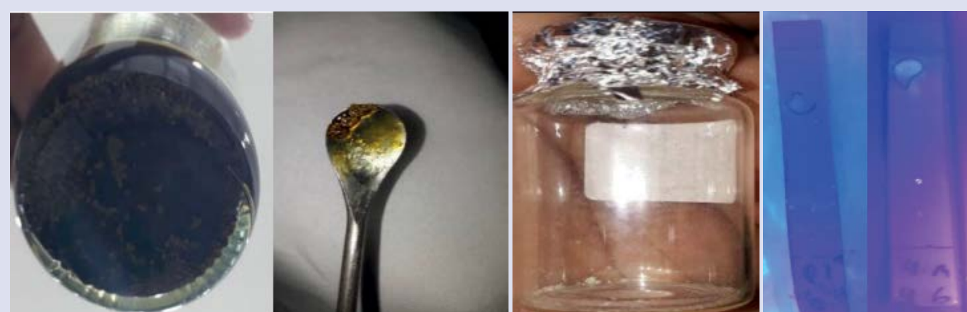

Figure 4. Isolate from the acetone fraction, (a) before recrystallization; (b) after recrystallization; (c) TLC results.

Figure 5. Isolate from the ethyl acetate fraction, (a) before recrystallization; (b) after recrystallization; (c) TLC results.

Anti-Inflammatory activities

In this anti-inflammatory research, the method used was the formation of artificial edema on the soles of mice's feet using carrageenan as an edema inducer.

Data from the Post Hoc LSD test results of the acetone fraction showed that all isolate and fraction comparisons had significant differences in per cent inhibition compared to the negative control. It seems that all comparisons of isolates and fractions are active as anti-inflammatory. It was also seen that there was no significant difference between the per cent inhibition of the 10 mg/KgBW fraction and the 5 mg/KgBW isolate. It indicates that several comparisons of fractions and isolates have the same inflammation inhibitory power. This similarity in inhibitory power occurs because the same chemical compounds may be

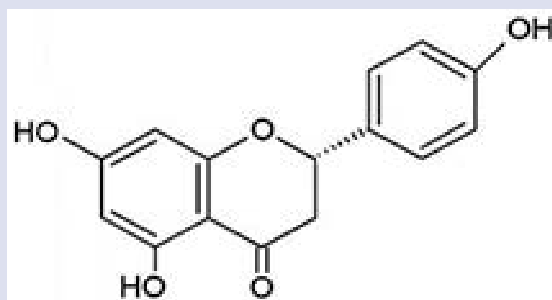

Figure 6. Structure of Naringenin[17].

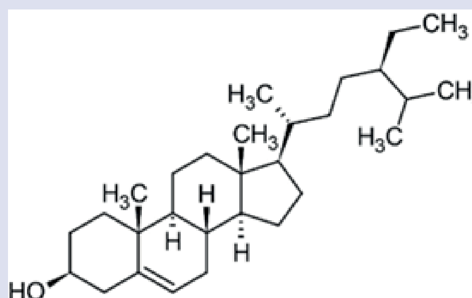

Figure 7. Structure of β-Sitosterol[18].

Table 4. Comparison of the IR spectra of Acetone and Ethyl Acetate isolates.

Acetone Fraction			
Isolate (cm^{-1})	Naringin (cm^{-1})	Naringenin (cm^{-1})	Functional Groups
3347.11	3398.53	3384	O-H polyhydroxy stretch
2880.64	-	2926	C-H aliphatic
1638.91	1626.21	1647	C=O stretch
1489	1588	-	C=C aromatics
1035.29	985.19	-	C-O-C stretch
Ethyl Acetate Fraction			
Isolat Isolate	β-sitosterol [15]	References[16]	Functional Groups
3254.05	3415.92	3549.99	O-H polyhydroxy stretch
2995.62	2945.30	2935.73	C-H aliphatic
1667.53	1649.14	1637.63	C=C Aromatic
1434.48	1454.33	1465.50	C-H (at $CH_{2)}$
1119.73	1047.35	1063.34	C-O Alcohol

Table 5. Post Hoc LSD Test Results in Testing Anti-Inflammatory Extracts.

Treatments	Doses (Kg/BW)	%Inhibition ± SEM
Na-Diklorofenak	10 mg	82.33 ± 0.245[a]
Acetone fraction	10 mg	47.67± 0.344[a,b]
	300 mg	55.67± 0.345[b,c]
	600 mg	67.33± 0.015[c]
	900 mg	79.00± 0.045[c]
Isolate of Acetone	3 mg	22.33± 0.145[b]
	5 mg	57.00± 0.005[b,c]
	10 mg	29.67± 0.025[b]
Ethyl acetate fraction	10 mg	53.37± 0.241[b]
	300 mg	75.51± 0.224[d]
	600 mg	67.72± 0.122c
	900 mg	83.40± 0.145[e]
Isolate of Ethyl acetate	3 mg	40.16± 0.125[b]
	5 mg	55.60± 0.115[b]
	10 mg	71.31± 0.045[c]

Tabel 6. Results of docking of test compounds with IFN-γ and IL-10 receptors.

Receptor	Ligand	Binding free energy (Kkal-mol^{-1})	Inhibition Constant (μM)	Hydrogen bonds	Hydrogen bond distance (Å)	Amino acid residues
IFN-γ	Naringenin	-1.71	56.08	GLU 157 PRO 158	2.31054 3.178	**ILE 160**, ASN 149, GLU 157, PRO 158
	β- Sitesterol	-6.58	15.01	-	-	**LEU 194, ILE 73, ALA 8, VAL 75**, ASP 73, **LEU 30**, LYS 34, LEU 33, ILE 97, PHE 74, VAL 5, TYR 4
	Native Ligand	-1.44	87.78	-	-	ILE 160, TRP 201, GLU 156, ASN 149, PRO 158, GLU 157
IL-10	Naringenin	-3.47	2.84	ASP 133 GLU 74	2.68407 2.0765	ASP 133, THR 136, GLU 74, **ILE 75, PRO 78**, ALA 79, **HIS 82, VAL 83,** ARG 137
	β- Sitesterol	--5.53	88.23	VAL 83	2.20874	**TYR 44,** ASP 43, ASP 42, ARG 137, PRO 85, VAL 83, TYR 84, THR 36, ARG 40, GLU 41
	Native Ligand	-1.39	95.87	ASN 134 GLU 129	1.74197 2.63498	ASP 133, GLU 129, ASN 134

Note: Amino acids in bold are hydrophobic interactions

present in several comparisons between the isolate and the fraction. The same thing applies to the ethyl acetate fraction; the post-hoc LSD test results for all doses have statistically significant differences in inhibition compared to the negative control. Values followed by different letter notations indicate a statistically significant difference.

The results of the above analysis on the Kolmogorov-Smirnov normality test stated that the data were normally distributed (P> 0.05) then continued with the homogeneity test, the significance was 0.937 > p (0.05) this indicates homogeneous data. Furthermore, the one-way ANOVA test was carried out, it was found that the statistical value was 0.001 > p (0.05), this means that there is a significant effect on the test group.

Molecular Docking Naringenin and β-Sitosterol

Receptor macromolecules used by IL-10 and IFN-γ files are saved in pdb format. Next, the macromolecules were optimized using AutoDockTools by adding hydrogen ions and Kollman charges and saved in pdbqt file format. The validation results measured the Root Mean Square Deviation (RMSD) values. The docking method is said to be valid if it has an RMSD value < 3 Å; this value indicates the protocol is accepted and docking can be carried out. The docking data displayed is in the form of binding affinity values and amino acid residue interactions. Docking results are saved in pdb format. The docking results and visualization are shown in Table 6 and Table 7.

DISCUSSION

This study used graded doses to determine the right amount that could show optimal anti-inflammatory effects. The effectiveness of the isolate and ethyl acetate fraction in reducing edema can be seen from the average percentage of edema. From Table 5, the 10mg/KgBW fraction has a significant portion of inhibition with Na-diclofenac 10mg/KgBW. The higher the concentration, the higher the per cent inhibition, and the ability to inhibit inflammation will also increase, indicating a high per cent inhibition at the optimum concentration.[19] It can be seen in testing anti-inflammatory extracts that have not been isolated. However, the anti-inflammatory activity will increase as the dose or concentration increases. However, in the 10mg/KgBW isolate, there was a decrease in anti-inflammatory activity. Several types of drugs in higher doses cause the release of histamine directly from mast cells, causing blood vessels to become more permeable to plasma fluid and causing an inflammatory process.[20] The test material can be anti-inflammatory if the inflammation inhibition value is above 50%. In the treatment of the test preparation with a concentration of 900 mg/KgBW, the fraction and isolate with 5mg/KgBW reached a higher percentage, namely 79.00% and 57.00%. It may be indicated to have anti-inflammatory effects. Naringin is widely used as a research compound because naringin can protect against inflammation in various experimental models, both in vivo and in vitro in animals.[17] Naringin is a flavonoid compound from the flavonone group that can act as an anti-inflammatory by inhibiting arachidonic acid and lysosomal enzyme secretion.[21] However, it has not achieved the positive control effect by administering Na-diclofenac 10mg/KgBW with an inflammation inhibition value of 82.33%. Naringenin know as a flavonoids with several bioactivities potential.[22] Naringenin is bound to a glycone to form the compound naringin.[17]

This similarity in inhibitory power occurs because there is the possibility of the same chemical compounds in the isolates and fractions, which is possible. After all, these fractions are in close order according to their separation in Vacuum Liquid Chromatography (VLC). However, fraction 2 has a larger average value for the per cent inhibition value. This shows that fraction 2 is more active when compared to the positive control; all doses given have significant differences in per cent inhibition values. Based on the test data, the differences between each treatment group can be seen. The greater the percentage value of inflammation inhibition, the greater the anti-inflammatory effect. The test material can be anti-inflammatory if the inflammation inhibition value is above 50%. With the ethyl acetate fraction at a dose of 900 mg/KgBW showing the highest inflammation inhibition value followed by a dose of 300 mg/KgBW, the inflammation inhibition value for both was not significantly different from control positive.

Naringenin is a flavonoid compound included in the flavanone subclass, which has biological activities in the form of antioxidant, antitumor, antiviral, anti-inflammatory, antiadipogenic and cardioprotective.[23] β- Sitesterol is the most abundant plant sterol in the human diet. β-Sitesterol can effectively fight prostate enlargement in clinical trials in humans and has anticancer and anti-inflammatory activity.[24] Naringenin and β-Sitesterol were subjected to a molecular docking test to predict anti-inflammatory properties using the IFN-γ and IL-10 receptors. From the results obtained in Table 1, the β-Sitesterol compound has the potential as an anti-inflammatory with the highest scoring value for IL-10 and IFN-γ receptors. The molecular docking test on β-Sitesterol with the IFN-γ receptor produced a binding energy value of -6.58 with an inhibition constant value of 15.01 μM. Meanwhile,

Table 7. Figure 2 Visualisasi 2D dan 3D.

Compounds	IFN-γ	IL-10
Naringenin		
β- Sitesterol		
Native Ligand		

the docking test of the β-Sitesterol molecule on the IL-10 receptor resulted in a binding energy value of -5.53 with an inhibition constant value of 88.23 μM and one hydrogen bond at VAL83 with a distance of 2.20874. β-Sitesterol is widely used in the pharmaceutical industry because of its unique biological and physicochemical properties.[25] In previous research, β-Sitesterol has various anti-inflammatory effects on peripheral tissues.[18]

From the scoring results, the test compound β-Sitesterol has tremendous potential as an anti-inflammatory than the compound naringenin. The β-Sitesterol compound has anti-inflammatory potential with IFN-γ and IL-10 receptors.[26] IFN-γ is a remarkably pleiotropic cytokine. It can enhance innate and adaptive immune responses against pathogens and tumours and maintain immune homeostasis. Because the effects of IFN-γ are cell and tissue-specific, it is essential to consider recent advances in IFN-γ signalling in the context of various diseases. To this end, we review the involvement of IFN-γ in the pathogenesis of several inflammatory diseases, its therapeutic potential as an antitumor agent and its effects on stem cells.[27] IL-10 is an anti-inflammatory cytokine inhibiting the pro-inflammatory response process in innate and adaptive immune cells. According to previous studies. [28] Interleukin 10 (IL-10) has strong anti-inflammatory properties that are central in limiting the immune response to pathogens, thereby preventing damage and maintaining normal tissue homeostasis.[28]

CONCLUSION

Our findings showed that the ethanolic (F1) and n-hexane (F2) fractions of P. canescens extract had good anti-inflammatory activity with per cent inhibition values of 58.12% and 56.59%, respectively. The characterization results showed that isolate F1 was a flavonoid group, Naringenin compound, while isolate F2 was a steroid group, β-Sitosterol compound. Furthermore, from the scoring docking results, β-Sitesterol has tremendous potential as an anti-inflammatory than the compound naringenin.

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