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Original Article

Isolation of a Flavone Apigenin and a Steroids Squalene from *Peronema canescens* Jack Leaves with Anti-Inflammatory Activities

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ABSTRACT

The leaves of the P. *canescens* are ethnobotanically used by the community as a remedy for bruises and fever. This study aims to isolate both of ethanol and n-hexane fractions of P. *canescens* Jack leaves. Isolate then characterized and determined their anti-inflammatory activity with male white mice. Anti-Inflammatory was determined by the volume of exudate and % inflammation inhibition. The isolates with good anti-inflammatory activity were characterized using UV-Vis and IR spectrophotometry. Our findings showed that the ethanolic (F1) and n-hexane (F2) fractions of P. *canescens* extract had a 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, Apigenin compound, while isolate F2 was a steroid group, Squalene compound.

Key words: P. *canescens,* Apigenin, Squalene, Anti-Inflammatory.

INTRODUCTION

Inflammation is inflammation as a complex immune response to foreign objects or foreign agents that enter the body.[1] Protective response in the form of pain, redness, swelling, and not functioning of tissues and organs as they should.[2] Although there is a natural process in the body, inflammation must be treated immediately, because if it is prolonged it can cause rheumatoid arthritis, atherosclerosis, fever, and ischemic heart disease.[3,4] Inflammation treatment can be done by taking drugs, such as non-steroidal drugs and steroid classes.[5,6] Steroid anti-inflammatory drugs work by inhibiting the formation of arachidonic acid, while non-steroidal anti-inflammatory drugs work by inhibiting the formation of prostaglandins. However, although these drugs have a strong anti-inflammatory effect, long-term use of these drugs will have dangerous side effects.[2,7] The high cost and the many side effects of synthetic drugs cause people to turn to traditional medicines using medicinal plants.[8,9]

Medicinal plants are one source that contains many efficacious compounds to treat various diseases.[10,11] Medicinal plants contain a lot of secondary metabolite compounds which basically have bioactive abilities.[12] One of the medicinal plants that grow in Indonesia and are widely used is the sungkai plant (*Paronema canescens* Jack). The leaves of the Sungkai plant are ethnobotanically used by the community as a remedy for bruises and fever.[13] Previous research has explained that sungkai leaf extract has antidiabetic activity,[14-17] Anti-inflammatory,[15] Antihyperuricemia,[14,16,17] and cytotoxic activity.[18] In addition, Sungkai leaf extract can also act as an antiplasmodial[19] and Antibacterial.[20-22] Most of the bioactive compounds having anti-inflammatory activity come from the alkaloid, phenolic and steroid groups. The ethanol extract of sungkai leaves has been studied to contain secondary metabolites of phenolic groups, flavonoids, tannins, alkaloids, steroids, and saponins.[15]

Research on the isolation of sungkai leaf bioactive compounds has not been explored in depth as an anti-inflammatory, in this study the aim was to partition the ethanol extract into n-hexane fraction and ethanol fraction as well as to test its anti-inflammatory activity with male white mice using the method of forming air sacs and artificial edema on the backs of mice. with carrageenan administered subcutaneously. In addition, isolates with good anti-inflammatory activity were characterized using UV-Vis and IR spectrophotometry.

MATERIAL AND METHODS

Material

The materials used in this study were *Peronema canescens* Jack leaves, EtOH, EtAce, n-hexane, silica gel (Merck), dichloromethane (DCM), acetone, thin layer chromatography plate on a plate covered with silica gel Merck 60 GF254 0, 25 mm, Vaseline flavum, Hydrocortisone acetate, 2% carrageenan, physiological NaCl, ethanol, Mg powder, concentrated HCl, FeCl3, Mayer's reagent (KgaA), Dragendroff's reagent (KgaA)), Aquadest, sulfuric acid, anhydrous acetic acid, Giemsa reagent (Sigma Aldric) and immersion oil. The equipment used in this study were maceration bottles, vacuum rotary evaporator, measuring cup, round bottom flask, beaker, separating funnel, column (Pyrex), stirring rod, watch glass, rope, roll meter, label paper, raffia rope, peg. , 1 ml injection syringe (One Med), 5 ml injection syringe (One Med), 3 ml injection syringe (One Med) analytical balance, animal scale, microscope, glass object, glass cover, experimental

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animal cage, UV-Vis (Thermo Scientific Genesys) and FTIR (Shimadzu IR Prestige).

Sample collection and preparation

P. canescens Jack leaves was collected from Kedemangan Village, Jambi Luar Kota District, Muaro Jambi Regency, Jambi Provinces, Indonesia. The samples were dried at room temperature and then ground into a coarse powder using an electrical grinder.

Extraction

P. canescens leaves were macerated in ethanol solution for 3 x 24 hours. The obtained macerate was then filtered using filter paper. The macerate was collected and evaporated with a vacuum vaporizer to obtain a thick extract. The thick extract was partitioned liquid-liquid with n-hexane as solvent using a separating funnel. The shaking was repeated until the color of the n-hexane solvent became clear. Then it was done again using ethyl acetate solvent until it was clear. There are three fractions obtained from the partition, namely the n-hexane, ethyl acetate, and ethanol fractions. Then each fraction is evaporated using a rotary evaporator so that it gets a thick fraction.[11]

In vivo animals study

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 hours. Before giving the treatment, they were given enough food and drink. During the experiment, food, and drink were placed ad libitum.[11]

Compound separation and purification

Thin Layer Chromatography (TLC). A TLC plate (1x5 cm) was prepared with a lower limit of 1 cm and an upper limit of 0.5 cm, so that the eluent distance is 3.5 cm. Furthermore, the eluent was made by comparing organic solvents with graded polarity. The extract was spotted on the lower border of the plate with a capillary tube, then eluted with the mobile phase/eluent. The shape of the stain was observed under a 254 nm UV lamp. Furthermore, the sample is impregnated using silica gel, then added to the column which already contains the stationary phase in the form of silica gel. While the mobile phase used is a solvent with a gradient polarity. The fraction obtained was accommodated in a vial. The results of the column chromatography were carried out by TLC again. The eluates that have identical stain patterns are combined based on the Rf value on the chromatogram.

Characterization of isolated compounds

Characterization of isolates using UV-Vis spectrophotometer using isolates as much as 2 ml were put in a cuvette and observed the spectrum at a wavelength of 200-800 nm. In addition, it was also characterized using an FTIR Spectrophotometer. 0.2 g of KBr pellet was added with 1 drop of isolate, dried, and then identified at wave number 4000-400 cm^{-1}.[16]

Anti-Inflammatory activities

Fraction and Isolate anti-inflamamtory activities were examined using carrageenan-induced paw edema and inflammation inhibition activity cotnnon pellet-induced granuloma in rats. antiinflamasi subcutaneously. Mice were inflamed using 2% carrageenan and then Vaseline flavum was used in the negative control and Hydrocortisone acetate 2.5% in the positive control. The extract was given with various concentrations of 55, 10%, and 15%. It was given continuously on days 5, 6, 7, and 8. For exudate volume measurement, it was carried out on day 9 where the mice were sacrificed by neck dislocation. The airbag on the back of the mice was opened and the volume of exudate was taken with a 1 ml syringe. Then the volume of exudate and the average percentage of inflammation inhibition were measured for each treatment.[16]

$$\%Edema = \frac{Vt-V0}{V0} \times 100\%$$

Vt (Final Volume)

V0 (Initial Volume)

Data analysis

Determination of the analysis of anti-inflammatory activity through the volume of exudate formed and the value of inflammation inhibition. All data were analyzed using one-way analysis of variance (ANOVA) for data that met the test of homogeneity and if there were differences, it was continued with the Tukey test.

RESULT AND DISCUSSION

P. cannescens leaf extract used three factions, n-hexane, ethyl acetate, and methanol fractions. The yield of the three fractions showed that the ethanol fraction had the highest yield (Table 1).

From the obtained fractions, phytochemical screening was carried out to obtain a profile of the secondary metabolites present in each fraction.

Secondary metabolite compounds contained in the ethanol fraction of P. *canescens* leaves are phenolic and flavonoid group compounds, while ethyl acetate contains steroids, alkaloids, and phenolics. In addition, n-hexane produces steroids and alkaloids.

Anti-inflammatory activity was tested using the *in vivo* method, granuloma sac formation. Mice were induced by carcinogenesis before being given treatment. Ethanol, n-hexane, and ethyl acetate fractions were used with concentrations of 5%, 10%, and 15%. A positive control used hydrocortisone acetate 2.5%, while the negative control used Vaseline flavum.

Isolation bioactive compound and Anti-Inflammatory activity

From each fraction, separation was carried out again using Gravity Column Chromatography (KKG) using an eluent gradient, starting from 100% n-hexane, n-hexane: ethyl acetate, 100% ethyl acetate, and ethyl acetate: ethanol.

The separation process is complete when the color of the eluate changes to clear. Separation of the ethanol fraction using Liquid Vacuum Chromatography produced 25 vials, then evaporated the solvent. From each vial, Thin Layer Chromatography (TLC) was performed to see the stain pattern of the components in the sample. The stationary phase used is a silica gel plate GF254 using a mobile phase in the form of a solvent ratio of ethyl acetate: ethanol (4:6). From each fraction, two significantly different fractions were produced. Furthermore, the four fractions were tested for anti-inflammatory using a concentration of 15%, this is to determine the value of the smallest exudate volume and the greatest inflammation inhibition of each fraction produced.

The results of the anti-inflammatory test showed that F1 (ethanol fraction) had a smaller exudate volume than fraction 2, while the F2 ethanol fraction was smaller with values of 56.6 L and 23.41 L,

Table 1: Percentage yield of ethanol extract of P. *canescens* Jack leaf extract.

Fraction	Method	% Yield
n-hexane (n-hex)	12.00	16.55
Ethyl acetate (EtAce)	18.99	26.19
Ethanol (EtOH)	28.70	39.58

Table 2: Profile of secondary metabolite compounds of P. *canscens* Jack.

Secondary Metabolites	Method	n-Hex	EtAce	EtOH	Reaction
Steroid	Lieberman Burchard	++	++	-	Blue or green color
Triterpenoids	Lieberman Burchard	-	-	-	Purple or orange color
Alkaloids	Drafendorf	++	++	-	Red precipitate, orange
Flavonoids	HCl + Mg	-	-	++	Foam formation, color
Phenolic	$FeCl_3$ 1%	-	++	++	Blackish purple color
Saponins	Foam test	-	-	-	Stable foam

++/Present

Table 3: Volume of exudate fraction.

Treatments	Dose (%)	Excudate Volume (µL)			
		I	II	III	Average ± SEM
Control (-)	2.5	155	150	155	$153,33^{d}$ ± 1,819
Control (+)	2.5	25	24	26	25.00^{a} ± 1,819
	5	115	116	114	115.00^{c} ± 1,819
EF	10	85	80	85	83.33^{b} ± 1.285
	15	55	50	55	55.33^{a} ± 1.33
	5	80.5	80	80	80.1^{e} ± 0.315
HF	10	68	66	66	66.67^{cd} ± 0.764
	15	46	47	42	45.00^{b} ± 1.082

EF: Ethanol fraction; HF: Hexane fraction
[a]Superscripts with different lowercase letters on the same line showed significant differences (Sig < 0.05)

Table 4: The Inflammation Inhibition

Treatments	Dose (%)	Excudate Volume (µL)			
		I	II	III	Average ± SEM
Control (-)	2.5	155	150	155	$153,33^{d}$ ± 1,819
Control (+)	2.5	82.14	86.67	82.35	83.72^{e} ± 1.476
	5	17.85	26.67	29.41	24.64^{b} ± 1,819
EF	10	39.28	50	44.11	44.46^{c} ± 1.285
	15	67.87	65.65	67.64	67.05^{d} ± 1.33
	5	19.5	20.0	30.0	19.83^{b} ± 0.167
HF	10	30.00	32.60	37.10	33.32^{c} ± 0.764
	15	51.00	53.6	60	54.78^{d} ± 1.082

EF: Ethanol fraction; HF: Hexane fraction
[a]Superscripts with different lowercase letters on the same line showed significant differences (Sig < 0.05)

Table 5: The exudate volume of isolate.

Treatments	Dose (%)	Excudate Volume (µL)			
		I	II	III	Average ± SEM
Control (-)	2.5	155.00	150.00	155.00	$153,33^{d}$ ± 1.29
Control (+)	2.5	25.00	24.00	26.00	25.00^{a} ± 1.19
EF	I1	60.00	55.00	55.00	56.60^{c} ± 1.29
	I2	100.00	95.00	95.00	96.67^{b} ± 1.285
HF	I1	63.30	55.55	61.70	60.00^{e} ± 0.315
	I2	43.00	43.00	44.00	43.33^{b} ± 0.764

Table 6: Percent inhibition of inflammation.

Treatments	Dose (%)	Excudate Volume (µL)			
		I	II	III	Average ± SEM
Control (-)	2.5 %	155	150	155	$153,33^{d}$ ± 1,819
Control (+)	2.5 %	25	24	26	25.00^{a} ± 1,819
EF	EI1 15%	71.43	50.00	52.94	58.12^{c} ± 1,819
	EI2 10%	25.57	28.00	26.17	26.58^{b} ± 1.285
HF	HI1 15 %	36.7	42.1	41.23	40.01^{c} ± 1.67
	HI2 10%	60.00	52.63	57.14	56.59 ± 2.145

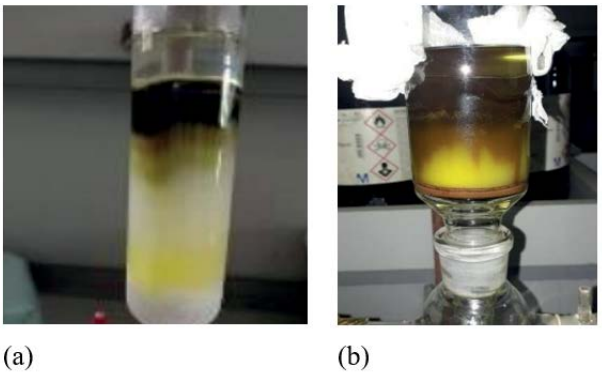

Figure 1: Compound solation using Liquid Vacuum Chromatography.

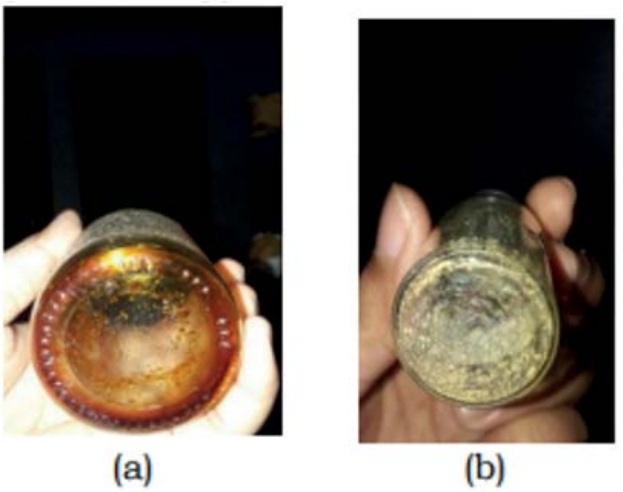

Figure 2: White crystal isolate of (a) EI1 and (b) HI2.

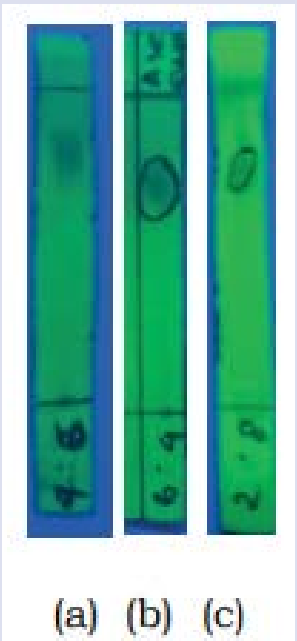

Figure 3: Isolate purity test using TLC; (a) EtAce: EtOH (4:6); (b) Acetone: EtOH (6:4); (c) DCM: Acetone (2:8).

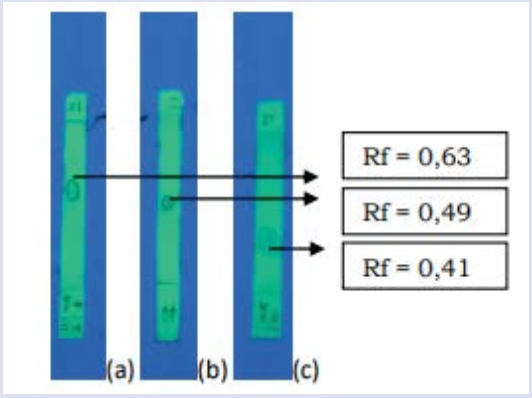

Figure 4: Isolate purity test using TLC; (a) DCM: Acetone (2:8); (b) n-Hex:EtAce (2:1); (c) DCM (100%).

respectively, and had inflammation inhibition values of 58, respectively. 12% and 56.59%. So it can be seen that fraction 1 has a strong anti-inflammatory compared to the other fractions. The results of the above analysis on the Kolmogorov-Smirnov normality test (Appendix 10) 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.

The results of the analysis carried out by the one-way ANOVA test consisting of one-way observations showed that the highest average volume of exudate was found in the negative control treatment and was followed by the fractional test preparations at concentrations of 5%, 10%, and 15% with the lowest volume average. in the positive control treatment. Giving ethanol fraction and n-hexane fraction of sungkai leaves to each group can be stated to have a significant effect (P> 0.05). To see the difference between the control and treatment groups, the Tukey test was carried out. The difference in each group is seen from the mean harmonic values obtained in each test group. The results of the analysis stated that the volume of exudate in the extract and fraction group at each concentration was lower than the negative control (C-) and significantly larger than the positive control (C+). In the negative control, the amount of exudate was greater than in the other test groups, this was due to the occurrence of an acute inflammatory process, namely fluid exudation of plasma proteins to the area where the inflammatory process occurred (Aria *et al*., 2015).

The inflammatory process due to cell injury occurs after being induced by carrageenan. The formation of edema manifests as a result of inflammatory mediators operating sequentially to produce an inflammatory response. Inflammatory mediators in the form of histamine will be released and there will be an increase in blood stimulation, protein fluid leakage in the tissue causes redness, increased pain, and swelling accompanied by pus (Lallo *et al*., 2020). Steroid compounds in the n-hexane fraction will have an anti-inflammatory effect by inhibiting the volume of edema in the area affected by inflammation and affecting the migration of the number of blood leukocytes and exudate in mice and can inhibit the activity of enzymes that play a role in the inflammatory process so that the process of inflammation widens in the inflammatory area not so (Amir *et al*., 2019). Steroid compounds are thought to reduce the volume of exudate. steroids work by inhibiting the release of the phospholipase enzyme from the source cells, by inhibiting the phospholipase enzyme so that arachidonic acid and prostaglandins are not formed by blocking the enzyme, stabilizing the lysosomal membrane, inhibiting the release of inflammatory mediators and migration and infiltration of leukocytes

In addition, the same result in the analysis of the average inflammation inhibition showed that the data were normally distributed (P>0.05).

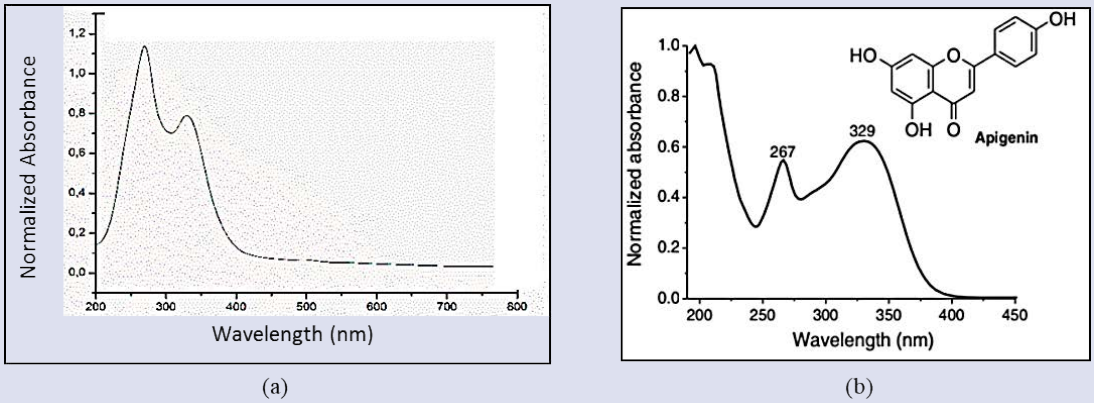

Figure 5: UV-Vis Spectrum of (a) EI1 and (b) Apigenin.

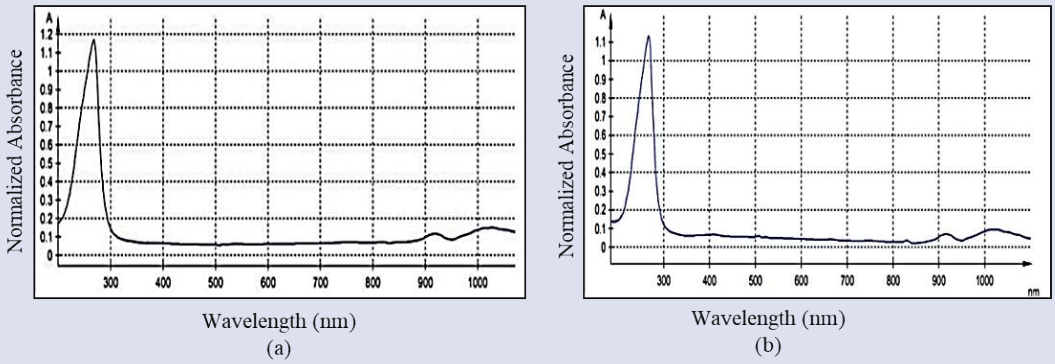

Figure 6: UV-Vis Spectrum of (a) HI1 and (b) Squalene.

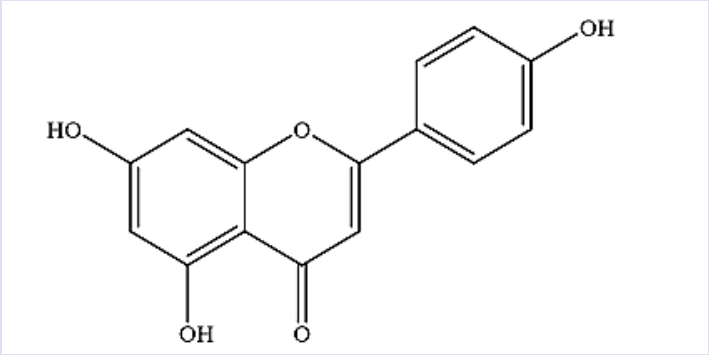

Figure 7: Chemical structure of Apigenin.

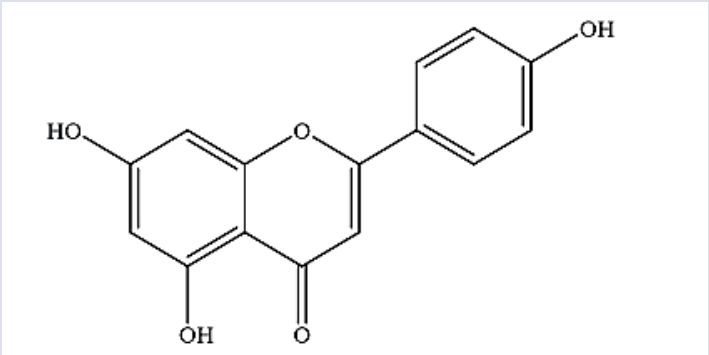

Figure 8: Chemical structure of Squalene.

To test the variation of the data obtained homogeneous variant data (P> 0.05). So the one away ANOVA test is valid. The results of the analysis stated that the treatment had a significant effect on each test group. Based on the test data, it can be seen the differences in each treatment group. The percentage of inflammation inhibition is defined as the ability to suppress the process of inflammation. The greater the percentage value of inflammation inhibition, the greater the anti-inflammatory effect. The test material can be said to have an anti-inflammatory effect if the inflammation inhibition value is above 50%.

Characterization of bioactive compounds

From the results of the exudate volume and percent inhibition test, it was found that isolates F1 in the ethanol fraction and isolates F2 in the n-hexane fraction had the best anti-inflammatory activity. So that the characterization of the two isolates continued.

Three different eluent systems were used to test the purity of pure isolates. Based on the purity test that has been carried out using a three-eluent system, it was found that there is a single stain, this indicates that the compounds contained in F1 and F2 are pure. Both isolates were screened for phytochemicals, and it was found that F1 was a flavonoid group while F2 was a steroid group.

Characterization using UV-Vis spectrophotometer

Characterization using UV-Vis spectrophotometer was carried out to determine the basic framework of the isolated compounds. From the results of characterization using UV-Vis spectrophotometer isolate EI1 obtained two maximum absorption peaks, namely band I at a wavelength of 329 nm and band II at a wavelength of 270 nm. The maximum absorption in band I of 329 nm is characteristic of the resonance of the sinamaoil group of ring B. The maximum absorption in band II of 270 nm is characteristic of the resonance of the benzoyl group of ring A (Indarto, 2015). The typical spectrum of flavonoids consists of two absorption maximums in the wavelength range of 230-295 nm (band II) and 300-560 nm (a band I).[23]

Based on the results of the characterization of F1 isolate compounds, it was found that F1 isolates were characteristic of flavonoid compounds of flavone and flavonol types. Flavone compounds have a maximum absorption region of band II in the range of 250-280 nm and a maximum area of band I in the range of 310-350 nm. Meanwhile, flavonol compounds have a maximum absorption area of band II in the range of 250-280 nm and band II in the range of 330-385 nm.[24] While the F2 isolate showed maximum absorption at a wavelength of 266 nm (1.1083 A) due to the electron transition from →π*, indicated the presence of a C=C chromophore which is a non-conjugated double bond. Compounds that have a conjugate system, have a smaller energy difference between the ground state and the excited state so that absorption occurs at a larger wavelength. Based on the comparison of the literature, the UV-Vis spectrum of the isolates has the same pattern with the UV spectrum of squalene, and the absorption bands are close to each other.

Characterization using FT-IR

Characterization of compounds using FT-IR spectrophotometer aims to identify the functional groups contained in compounds based on specific wave numbers. The results of the comparison of isolates to similar compounds show that there are similarities in functional groups (Table 8 and Table 9).

The FT-IR spectrum of isolate F1 obtained has a similar pattern to the comparison FT-IR spectrum obtained by comparing it with previous studies. This is reinforced by the interpretation of the data on the functional groups identified in F1 isolates are the same as apigenin. The wave number 3338 cm^{-1} with sharp intensity indicates the stretching vibration of the O-H group. This is reinforced by the absorption at wave number 2945 cm^{-1} which indicates the CH_3 group and wave number 2836 cm^{-1} which is aliphatic CH_2 absorption. A wave number of 1657 cm^{-1} indicates the presence of a C=O ketone group. Wave number 1543 cm^{-1} shows ring C=C. At wave number 1456 cm^{-1} is C-O-H stretching vibration. At a wave number of 1024 cm^{-1} with sharp intensity, it indicates the presence of cyclic C-O-C. Based on the results of the FT-IR spectrophotometer characterization of F1 isolates, it was suspected that F1 isolates were compounds of the flavonoid group. The allegation is reinforced by the literature FT-IR spectrum data that isolates apigenin which is a flavonoid compound of the flavone type from marine red algae. The comparison of FT-IR spectrum data can be seen in Table 8.

Table 7: Isolate phytochemical screening.

Secondary Metabolites	Method	Isolate EI	Isolate HI	Reaction
Steroid	Lieberman Burchard	-	++	Blue or green color
Triterpenoids	Lieberman Burchard	-	-	Purple or orange color
Alkaloids	Drafendorf	-	-	Red precipitate, orange
Flavonoids	HCl + Mg	++	-	Foam formation, color
Phenolic	$FeCl_3$ 1%	-	-	Blackish purple color
Saponins	Foam test	-	-	Stable foam

Table 8: Comparison of the IR spectrum of isolates F1 and Apigenin.

Wave Number (cm^{-1})		Functional group
Isolate F1	**Apigenin**[29]	
3338	3333	O-H stretch
2945	2946	C-H Aliphatic (stretch)
1657	1646	C=C (alkene)
1543	1578	C=C
1456	1466	C-OH stretch
1024	1024	C-O stretch

Table 9: Comparison of the IR spectrum of isolates HI2 and Squalene.

Isolat HI2	Wave Number (cm^{-1})	Functional groups
	Derivated Squalene[10]	
3351,11	3321,82	O-H
2959,61	2939,22	C-H aliphatic (Stretch)
1756,72	1643	C=C (alkene)
1455,08	1512,19	C-H aliphatic (bend)
1455,08	1021,24	C-O alcohol

Based on the IR spectrum of the F1 isolate, the n-hexane fraction of Sungkai leaves showed absorption of strain vibrations from O-H in the 3351.11 cm^{-1} area of the widened band. At a wave number of 2959.61 cm^{-1}, it shows the stretching vibration of the aliphatic C-H group, and the area of 2877.02 cm^{-1} shows the $-CH_3$ group. In the area of 1756.72 cm^{-1}, there is a C=C functional group. The absorption at the wave number of 1017.34 cm^{-1} contained a C-OH functional group due to C-O stretching in the 1050-1200 cm-1 area. The presence of 3351.11 cm-1 stretches indicates the O-H functional group and C-O vibrations at the peak of 1017.34 cm^{-1} support the presence of steroid compounds in the isolate. At the peak of the aliphatic C-H stretch in the area (2959.61 cm-1 and 2877.02 cm^{-1}) and the C=C group (1756.72 cm^{-1}) from the characterization results on isolate F1.3, it was suspected that squalene compounds were present. Squalene compounds have anti-inflammatory properties that can reduce redness and swelling. The mechanism of action of squalene compounds is by increasing macrophages. Macrophages are a type of white blood cell that plays a role in the immune system and can help the wound healing process faster.[10]

Apigenin, a 4',5,7 trihydroxy flavone, belongs to flavone subclass of flavonoids. It is ubiquitously distributed in leaves, vegetables, stems, and fruits of several plants.[25] Based on previous studies apigenin known has a potent antioxidant, cyclooxygenase inhibitor, cell cycle inhibitor, protein kinase C inhibitor, and apoptosis inducer. Apigenin belongs to Flavonoids which are a multi-functional group that possess substantial characteristics that can be exploited for the development of therapeutic agents targeting several chronic diseases. They have been seen to exert a wide range of pharmacological effects, such as anti-oxidant, anti-tumor, anti-viral, anti-allergic, anti-inflammatory, and anti-viral effects. These protective biological properties are mostly due to the phenolic structure of these flavonoids.[26]

Squalene, a 2,6,10,15,19,23-hexamethyl tetracosahexaene known belongs to a natural lipid, which contain six isoprene units, and a biochemical precursor of cholesterol biosynthesis and other biolocical substances.[27] Previous studies report that squalene is a highly effective antioxidant acting as a direct reactive oxygen species (ROS) scavenging agent reducing intracellular oxidant stress and also protects human skin surfaces from lipid peroxidation as a quencher of singlet oxygen.[28]

CONCLUSION

Our findings 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, Apigenin compound, while isolate F2 was a steroid group, Squalene compound.

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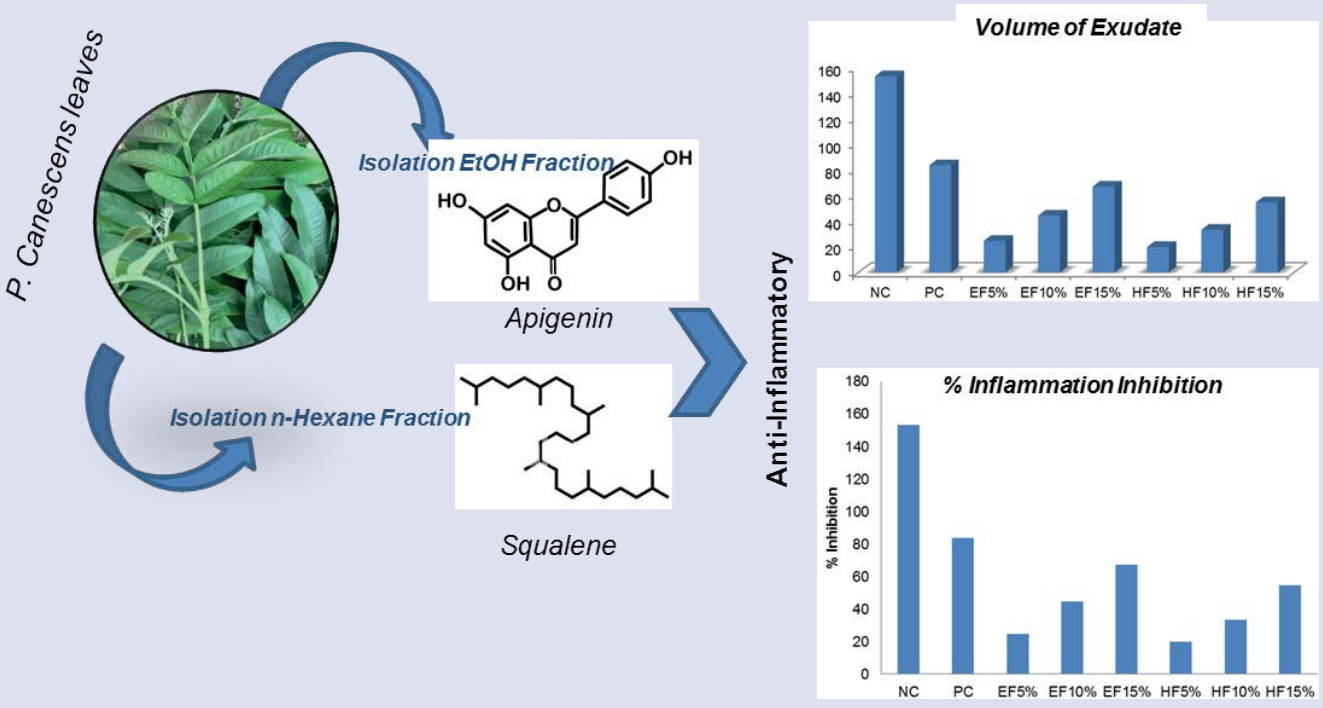
GRAPHICAL ABSTRACT



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