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THE ANTICHOLESTEROL ACTIVITY OF BETULINIC ACID AND STIGMASTEROL ISOLATED FROM THE LEAVES OF SUNGKAI (*PARONEMA CANESCENS* JACK)

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ABSTRACT

Objective: The purpose of this study was to evaluate the anticholesterol activity of *Paronema canescens* leaves extract.

Methods: These compounds were isolated via chromatography using silica gel as the stationary phase. Their structures were revealed using IR and 1D NMR data and by comparing their NMR data with the literature's data. The anticholesterol activity of compounds was tested using photometry via the Liebermann-Burchard reaction.

Results: Betulinic acid (1) and stigmasterol (2) were isolated from the leaves extracts of *sungkai* (*P. canescens*). The betulinic acid (1) that was isolated showed anticholesterol activity with an IC₅₀ value of 60.53 µg/ml, stigmasterol (2) 222.32 µg/ml, while the standard anticholesterol compound simvastatin had an IC₅₀ of 24.68 µg/ml.

Conclusion: The stigmasterol (2) has previously been reported from *P. canescens* while that of betulinic acid (1) is reported here for the first time and identifies its anticholesterol activity.

Keywords: *Paronema canescens*, Betulinic acid, Anticholesterol

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INTRODUCTION

P. canescens is commonly known as *sungkai* or *jati barang*. In Indonesia, this plant is found in South Sumatra, Lampung, Bengkulu, and Kalimantan. This plant's leaves have been used traditionally to cure fever, malaria, wounds, ringworm, and toothaches [1, 2]. The water from the boiled stem bark of *sungkai* is used as a smallpox medicine [3]. In South Sumatra, Indonesia, the people of Musi Banyuasin use this plant to treat hypertension and high cholesterol (hyperlipidemia) [4]. Hypertension is one of the most common diseases in the world. One of the triggers for hypertension is a high level of cholesterol in the blood. Cholesterol is a fatty lipid produced by the liver and is crucial for normal body functioning. The cholesterol level in blood was influenced by several factors like nutrition, diet, weight, and including drugs consumed [5]. The buildup of cholesterol in the blood can cause the narrowing and stiffness of blood vessel walls, causing blood pressure to increase. Hypertension can lead to coronary heart disease and stroke [6, 7].

Several chemical contents and biological activities of *P. canescens* have been reported in the literature. The methanol extract from the leaves of *P. canescens* has been reported to have antibacterial activity [8]. The methanol extract from the leaves of *P. canescens* also contained alkaloid, terpenoid, steroid, flavonoid, and tannin [9]. The acetone extract from the leaves of *P. canescens* was reported to contain seven clerodane-type furanoditerpenoid compounds: peronemins B2, A2, B3, A3, B1, C1, and D1, and caffeine acid glycosides were shown to have antimalarial activity. The acetone extract from the leaves of *P. canescens* has also been found to contain β-sitosterol, phytol, β-amyrin, peronemin, isopropanol, diterpenoids, acetonide, and flavonoid glycoside [10]. The methanol extract from the leaves of *P. canescens* was reported to have antibacterial activity. The leaves of *P. canescens* were also said to have weak antiplasmodial and cytotoxic activity [11] and to be useful as insecticides. However, no chemical studies have been reported regarding the anticholesterol activity of the leaves of *P. canescens*. Phytochemical test of the ethanolic extract of the *sungkai* plant was positive for alkaloid, flavonoid, steroids, triterpenoid, glycoside, and phenol [12]. Steroid, terpenoid, and phenol effect of reducing cholesterol level.

Therefore the purpose of this study was to evaluate the anticholesterol activity of *P. canescens* leaves extract.

MATERIALS AND METHODS

General experimental procedures

The IR (KBr) spectrum was recorded on a Perkin Elmer-FTIR spectrometer. NMR spectra were recorded on an NMR JEOL JNM ECA-500 spectrometer (500 MHz for ¹H NMR and 125 MHz for ¹³C NMR spectra) in CD₃OD or CDCl₃. UV-visible spectrophotometry using a single beam was done with the Shimadzu-UV mini 1240 instrument. Column chromatography was performed with silica gel 60G (70–230 mesh and 230–400 mesh). Thin-layer chromatography was performed with silica gel 60G F₂₅₄ using Merck (Art.5554), and spots were visualized by spraying with cerium sulfate vapours and ultraviolet light. Only analytical reagent grade solvents were used.

Sample collection

The fresh leaves of *P. canescens* were collected from the Musi Banyuasin Regency of South Sumatra, Indonesia in October 2019. The plant was identified as *P. canescens* Jack by Dr. Laila Hanum (Voucher specimen VIC 2704), head of the Botany Laboratory, University of Sriwijaya. The plant was deposited at the Botany Laboratory in the Biology Department at the University of Sriwijaya.

Extraction and isolation

The fresh leaves of *P. canescens* (1.2 kg) were extracted using the maceration method with step gradient polarity and using the solvents *n*-hexane and ethyl acetate. The leaves soaked for 3 d before filtration. This process (of maceration and filtration) was repeated three times. The filtrate was then concentrated using a vacuum rotary evaporator at about 60 °C and obtained a crude *n*-hexane extract and ethyl acetate extract [13]. The crude *n*-hexane extract (15.97 g) was separated using vacuum column chromatography with silica gel 60G (230–400 mesh) as the stationary phase. Samples were prepared by pre-adsorption using silica gel 60G (70–230 mesh) and dried to form a powder. The sample was eluted with solvent in increasing proportions of ethyl acetate in *n*-hexane 10:0 (300 ml),

9:1 (400 ml), 8:2 (400 ml), 5:5 (300 ml), 4:6 (300 ml), and 0:10 (300 ml). The eluent was placed in bottles (500 ml). Each bottle was analyzed using thin-layer chromatography (TLC) using a mobile phase with a 7:3 ratio of *n*-hexane to ethyl acetate. Eluents that showed the same TLC patterns were combined and concentrated to a minimum volume and obtained the fractions F1 (1.64 g), F2 (1.22 g), F3 (1.35 g), F4 (2.88 g), and F5 (2.49 g). The F4 fraction (3.25 g) was separated and purified using open column chromatography and silica gel 60G (70-230 mesh) as the stationary phase [14]. Samples were also prepared by pre-adsorption. The column was first eluted with *n*-hexane and then with the solvent that was prepared by gradually increasing the percentage of ethyl acetate in *n*-hexane. The ratios of *n*-hexane to ethyl acetate that were used: 9:1 (300 ml), 8:2 (400 ml), 7:3 (100 ml), 6:4 (150 ml), 5:5 (100 ml), and ethyl acetate 100% (300 ml). Eluates were collected in bottles (10 ml), and each bottle was analyzed using TLC. The eluates that showed the same TLC patterns were combined and concentrated to a minimum volume and four subfractions F4.1 (0.339 g), F4.2 (0.520 g), F4.3 (0.225 g), and F4.4 (0.539 g) were obtained. The F4.3 fraction indicated the formation of a white solid, and after purification with ethyl acetate, obtained a white amorphous solid 1 (8.5 mg).

The ethyl acetate extract (30 g) was then separated using vacuum column chromatography with increasing ethyl acetate proportions in *n*-hexane (10:0-0:10) as mobile phase, each with a volume of 300 ml. The eluents that showed the same TLC pattern were pooled and concentrated to a minimum volume and obtained three fractions F1 (4.72 g), F2 (3.27 g), and F3 (17.18 g). The F2 (3.27 g) fraction was separated using open column chromatography with silica gel G60 (70-230 mesh) as the stationary phase. Solvents were made by increasing concentrations of ethyl acetate in *n*-hexane. The ratios of *n*-hexane to ethyl acetate that were used: 10:0 (200 ml), 9:1 (200 ml), 8:2 (300 ml), 5:5 (300 ml), and ethyl acetate 100% (300 ml). Eluates were analyzed using TLC. Eluates that showed combined the same TLC pattern, and obtained five subfractions F2.1 (0.325 g), F2.2 (0.855 g), F2.3 (0.112 g), F2.4 (0.720 g), and F2.5 (1.02 g). Fraction F2.3 was a greenish-white solid, and after recrystallization in *n*-hexane, obtained a white amorphous solid 2 (12 mg). The compound that was isolated was analyzed using TLC with eluents with different polarities, and spots were visualized by spraying with cerium sulfate vapour to verify that it was a pure compound. Furthermore, IR and 1D NMR spectroscopic analysis was performed on the compounds that were isolated.

Anticholesterol activity

The anticholesterol activity of the compounds was determined using photometry. Cholesterol reacts with the Liebermann-Burchard reagent [15]. Simvastatin was used as a positive control. The concentrations of the compounds that were isolated and simvastatin that were used: 10, 20, 30, 40, 50, 75, and 100 µg/ml. Samples (2.5 ml) were tested by the addition of 2.5 ml cholesterol standard 100 µg/ml and then shaken for 2 min and combined with 2 ml of acetic anhydride and 0.1 ml of sulfuric acid. Left the mixture was left for 16 min at room temperature. The measurements were done three times. As a control, they were using cholesterol 100 µg/ml. The absorbance data measured at λ_{max} 420 nm. Calculated the percentage of inhibition was calculated with the formula:

$$\% \text{ Inhibition} = \frac{A_o - A_s}{A_o} \times 100$$

Ao: absorbance control and As: absorbance sample

RESULTS AND DISCUSSION

Extraction of fresh leaves of *P. canescens* (1.2 kg) with a solvent (*n*-hexane and ethyl acetate) and evaporated to dryness under a high vacuum at about 60 °C, produced a crude *n*-hexane extract (15.97 g) and ethyl acetate (62.69 g), with yield percentages 1.33% and 5.22% respectively. The yield percentages demonstrated that the ethyl acetate fraction was greater in quantity than the *n*-hexane fraction because ethyl acetate is a semi-polar solvent that can attract nonpolar and polar compounds. The *n*-hexane extract (15.97 g) was separated and purified using a chromatography column to obtain a white, amorphous solid (1) (8.5 mg). The ethyl acetate extract was separated and purified to obtain a white amorphous solid (2) (12

mg). Performed spectroscopic analysis (IR and NMR 1D) to determine the compound's molecular structures that were isolated. FTIR spectrometer was recorded on between the range 3500-1000 cm⁻¹. The wavelength of light absorbed is characteristic of the chemical bonds [16]. The IR spectrum for the compound (1) had absorption bands at 2966 cm⁻¹ and 2880 cm⁻¹ (C-H aliphatic stretching in the form of CH₃, CH₂ and CH groups) and absorption frequencies at 1156 cm⁻¹ (C-O) and 1774 cm⁻¹ (C=O acid). The IR spectrum also had absorption bands at 1661 cm⁻¹ (C=C isolated), 1468 cm⁻¹ (cyclic (CH₂)_n bending), and 1392 cm⁻¹ (gem-dimethyl-CH(CH₃)₂). The IR spectrum of the compound had several vibrations characteristic of the triterpenoid compound.

The ¹H NMR spectrum of the compound (1) (fig. 1) showed no absorption in the δ_H 6-8 ppm region, which indicated that the compound was cycloaliphatic and a number of the terpenoid group. The shielded region of the spectrum indicated overlapping resonances for methylene and methine protons. ¹H NMR (CD₃OD, 125 MHz) δ_H ppm values and ¹³C NMR (500 MHz) δ_C ppm values of the compound is shown in table 1. The ¹H NMR spectrum of the compound showed resonances for methylene olefinic protons at δ_H 4.71 (1H, *d*, *J* = 2.5 Hz) and 4.59 (H, *d*, *J* = 2.5 Hz). The peak at δ_H 4.62 (1H, *s*) was the H bond signal to the oxygen atom (OH). The proton ¹H NMR spectrum showed the proton H-3 appeared at δ_H 3.13 ppm (1H, *m*) as an oxymethine proton. Six singlet signals with strong intensities with chemical shift values of δ_H 0.75 (3H, *s*), 0.86 (3H, *s*), 0.95 (3H, *s*), 0.96 (3H, *s*), 1.00 (3H, *s*), and 1.69 (3H, *s*) that are characteristic signals of methyl proton in triterpenoid were also obtained. The ¹H NMR spectrum methyl proton signal at δ_H 1.69 ppm is the typical signal for methyl allylic proton [17]. The ¹H NMR spectrum of compound 1 contained only six methyl groups instead of eight, which is expected for a triterpene. Since converted one of the methyl group into an olefin, the remaining methyl group must have been oxidized into a carboxylic acid. This was confirmed using the ¹³C NMR spectrum of the compound (table 1), which resonated with the carboxylic acid at δ 180.2 ppm. These resonances are typical of triterpenes with olefin, hydroxyl, and carboxylic acid functional groups but did not detect the carboxylic acid proton in the ¹H NMR spectrum.

The ¹³C NMR spectrum (in CD₃OD) (fig. 2) showed 30 carbon signals (table 1). Resonance for the oxymethine carbon (δ_C 79.8), which is commonly found in triterpenoid compounds, and olefinic carbons (δ_C 152.1 and δ_C 110.4) were also detected (fig. 2). The remaining 26 carbon resonances were attributed to the methyl, methylene, methane, and quaternary carbons. The DEPT spectrum, the compound showed seven quaternary carbon signals with chemical shifts at δ_C 180.2, 152.1, 38.4, 42.0, 40.0, 39.8, and 57.6 ppm; five-carbon methine signals at δ_C 79.7, 56.9, 52.1, 50.2, and 48.6 ppm; 11 methylene signals at δ_C 38.2, 28.1, 19.6, 35.7, 22.2, 26.9, 30.9, 31.8, 33.4, 40.2, 110.3 ppm; and six methyl signals at δ_C 28.7, 19.5, 16.2, 16.8, 16.7, and 15.2 ppm.

The NMR spectrum data for compound 1 was compared with data in the literature. Based on the literature, the ¹H-NMR and ¹³C-NMR data of the compounds show great similarities with betulinic acid, as shown in table 1 [18]. Based on the IR, ¹H NMR, and ¹³C NMR spectroscopic data analysis, and by comparing with the data in the literature, compound 1 found to be betulinic acid (fig 3). This is the first report on the isolation of the compound from *P. canescens*. Betulinic acid is a pentacyclic triterpene found in many plant species including in the bark of *Dillenia kerrii* [19] and *Dillenia papuana* [20]. Betulinic acid has also been found in *Dillenia philippinensis* and has active antibacterial properties [17]. Betulinic acid has also been reported to display various biological activity, including anticancer activity [21] and anti-HIV agent [22, 23].

Compound 2 was a white amorphous solid. The IR spectrum of compound 2 in the KBr pellet showed some characteristic absorptions at 3410 cm⁻¹ (OH group), 2935 cm⁻¹, and 2868 cm⁻¹ (aliphatic stretching-CH asymmetric and symmetric). Other peaks were seen at 1624 cm⁻¹ (C=C), 1480 cm⁻¹, and 1377.17 cm⁻¹ (cyclic bending of (CH₂)_n and (CH₃)₂CH). There were also peaks at 1043 cm⁻¹ (C-O alcoholic) and 970 cm⁻¹ (unsaturated-CH vibration). The ¹H NMR chemical shifts at δ_H 5.35 (1H, *d*, *J* = 6.0 Hz), 5.05 (1H, *dd*, *J* = 8.5, 15 Hz), and 4.95 (1H, *dd*, *J* = 8.5, 15 Hz) were the three

olefinic proton shifts characteristic of stigmasterol. The chemical shifts at δ_H 3.32 ppm (1H, *m*) indicated oxymethine protons, which is characteristic of steroid compounds that contain OH groups bound to C-3. The 1H NMR spectrum also showed a strong intensity signal that are characteristic signals of methyl protons. The doublet signal

at 0.81, 0.83, 0.85 ppm and integration of three protons for signal methyl. This is a characteristic signal for methyl groups in stigmasterol compound. The two singlet signals from methyl atom at δ_H 0.73 and 1.02 ppm (3H, *s*) are characteristic signals for the methyl position C-18 and C-19 in stigmasterol.

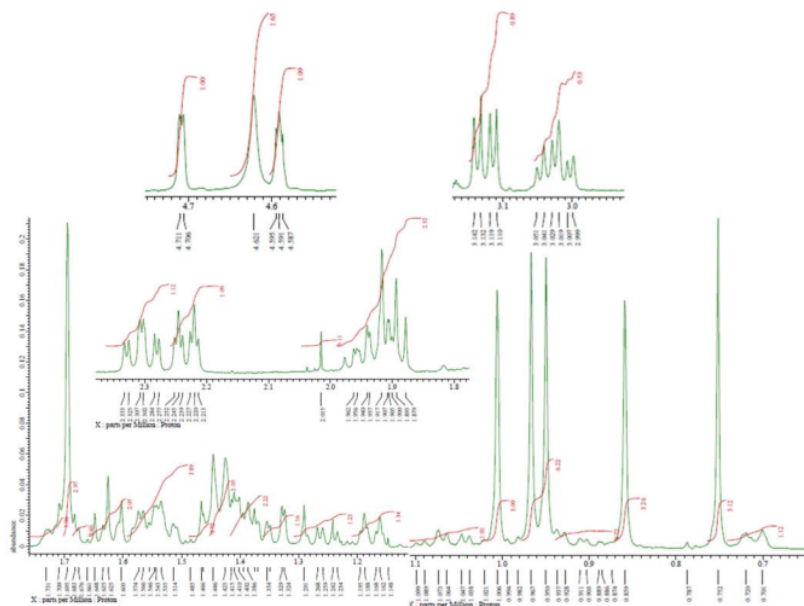
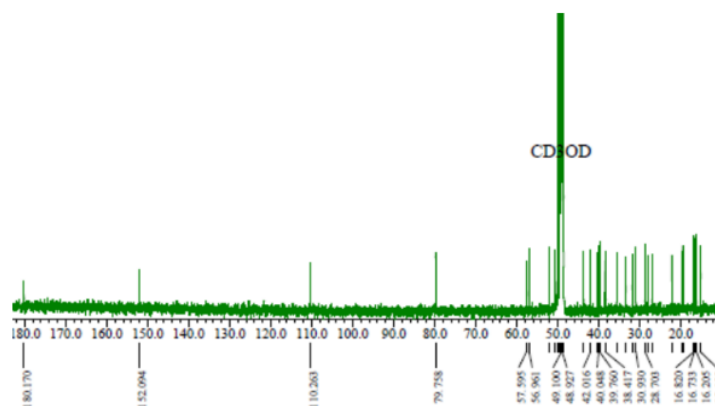


Fig. 1: 1H NMR Betulinic acid (1)

Table 1: 1H and ^{13}C NMR spectral chemical shift values for compound 1 (1H -500 MHz, ^{13}C -125 MHz, in CD_3OD) and betulinic acid [18] (1H -400 MHz, ^{13}C -100 MHz, in $CDCl_3$, ppm)

C	δ_C [18]	δ_C 1	δ_H mult. (J Hz)[18]	δ_H , mult. (J Hz) 1	PT
1	38.7	38.2	0.90, 1.69		CH ₂
2	27.4	28.1	1.55, 1.62		CH ₂
3	79.0	79.7	3.20 <i>dd</i> (4.5, 11.5)	3.13 (<i>dd</i> , 5.0, 10.5)	CH
4	38.9	38.4	-		C
5	55.3	56.9	0.68		CH
6	18.3	19.6	1.53		CH ₂
7	34.3	35.7	1.40		CH ₂
8	40.7	42.0	-		C
9	50.5	52.1	1.26		CH
10	37.2	40.0	-		C
11	20.8	22.2	1.41, 1.43		CH ₂
12	25.5	26.9	1.66, 1.68		CH ₂
13	38.4	39.8	2.20		CH
14	42.4	43.7	-		C
15	29.7	30.9	1.22, 1.55		CH ₂
16	32.1	31.8	1.42, 2.30		CH ₂
17	56.3	57.6	-		C
18	49.3	50.2	1.62		CH
19	46.9	48.6	3.02 <i>dt</i> (10.5, 5.5)	3.02 (<i>m</i>)	CH
20	150.4	152.1	-		C
21	30.5	33.4	1.41, 1.99		CH ₂
22	37.0	40.2	1.48, 1.96		CH ₂
23	27.9	28.7	0.95 (<i>s</i>)	0.96 (<i>s</i>)	CH ₃
24	15.3	16.2	0.74 (<i>s</i>)	0.75 (<i>s</i>)	CH ₃
25	16.1	16.8	0.80 (<i>s</i>)	0.86 (<i>s</i>)	CH ₃
26	16.0	16.7	0.92 (<i>s</i>)	0.95 (<i>s</i>)	CH ₃
27	14.7	15.2	0.96 (<i>s</i>)	1.00 (<i>s</i>)	CH ₃
28	179.8	180.2	-		C
29	109.7	110.3	4.62 <i>d</i> (2.0), 4.70 <i>d</i> (2.0)	4.71 <i>d</i> (2,5), 4.59 <i>d</i> (2,5)	CH ₂
30	19.4	19.5	1.69 (<i>s</i>)	1.69 (<i>s</i>)	CH ₃

Fig. 2: ^{13}C NMR betulinic acid (1)

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Table 2: ^1H and ^{13}C NMR spectral chemical shift values (ppm) for compound 2 (^1H -500 MHz, ^{13}C -125 MHz), and stigmasterol (^1H -400 MHz, ^{13}C -100 MHz, in CDCl_3 , ppm) [24]

C	δ_c stigmasterol [24]	δ_c 2	δ_H (ppm), ΣH , mult. (J Hz), stigmasterol [24]	δ_H (ppm), ΣH , mult. (J Hz), 2	DEPT
1	37.3	38.6			CH_2
2	28.3	29.4			CH_2
3	71.9	72.5	3.52 (1H, <i>dd</i>)	3.32 (1H, <i>m</i>)	CH
4	42.4	43.1			CH_2
5	140.8	142.3			C
6	121.7	122.3	5.34 (1H, <i>d</i>)	5.35 (1H, <i>d</i> , 6.0)	CH
7	31.7	33.1			CH_2
8	34.0	33.3			CH
9	50.2	51.8			CH
10	36.5	37.7			C
11	26.2	27.2			CH_2
12	39.8	41.2			CH_2
13	42.4	43.5			C
14	56.8	57.5			CH
15	24.3	24.2			CH_2
16	29.2	32.3			CH_2
17	56.1	58.2			CH
18	12.0	12.5	0.67 (3H, <i>s</i>)	0.73 (3H, <i>s</i>)	CH_3
19	19.4	19.4	1.00 (3H, <i>s</i>)	1.02 (3H, <i>s</i>)	CH_3
20	40.5	37.5			CH
21	21.1	20.3			CH_3
22	138.3	139.9	5.16 (1H, <i>dd</i> , 8.4, 15)	5.05 (1H, <i>dd</i> , 8.5, 15)	CH
23	129.0	130.0	5.03 (1H, <i>dd</i> , 8.4, 15)	4.95 (1H, <i>dd</i> , 8.5, 15)	CH
24	51.3	52.9			CH
25	45.9	47.3			CH
26	19.4	19.5	0.81 (3H, <i>d</i>)	0.83 (3H, <i>d</i>)	CH_3
27	19.8	19.9	0.79 (3H, <i>d</i>)	0.81 (3H, <i>d</i>)	CH_3
28	24.3	25.4			CH_2
29	12.3	12.4	0.85 (3H, <i>d</i>)	0.86 (3H, <i>d</i>)	CH_3

The ^{13}C NMR spectrum showed four signals above 100 ppm (142.3, 139.9, 130.0, and 122.5 ppm). The signals at δ_c 142.3 and 130.0 ppm were characteristic of C-22 and C-23 in stigmasterol [24]. The presence of four sp^2 hybridized carbon signals indicated that the isolated compound had two double bonds. The ^{13}C NMR spectrum also showed a signal at δ_c 71.3 ppm, which is the signal for sp^3 hybridized carbon, a typical signal for C-3 atoms. The ^{13}C NMR spectrum for compound 2 showed 29 carbon signals. DEPT spectrum was used to determine the type of carbon (methyl, methylene, methine or quaternary carbon). The DEPT spectrum revealed six methyl groups at δ_c 12.4, 12.5, 19.4, 19.5, 19.9, and 20.3 ppm; nine methylene groups at δ_c 24.2, 25.5, 27.2, 29.4, 32.2, 33.1, 38.6, 41.2, and 43.1 ppm; 11 methine groups at δ_c 72.5, 130.0, 139.9, 122.3, 51.8, 52.9, 57.5, 58.2, 47.3, 37.5, and 33.3 ppm; and three quaternary carbons at δ_c 142.3, 37.7, and 43.5 ppm.

The NMR data of compound 2 was compared with data in the literature (table 2). The compound's chemical shift values were almost identical to those of the ^{13}C NMR spectrum of the stigmasterol compound found in the literature [24]. The Chloroform was used as the solvent for the measurements of both the compound and stigmasterol. The little difference in the value of the chemical shifts seen in the spectrum for the compound that was isolated and for stigmasterol found in the literature is probably due to the differences in the instrumentation used. The ^{13}C -NMR spectral measurement of the compound was isolated at 500 MHz, while that of stigmasterol was done at 400 MHz [24]. Based on the analysis of spectroscopic data IR and ^1H NMR spectral data, and using data found in the literature for stigmasterol, the compound 2 was found to be stigmasterol. Its structure is shown in fig. 3.

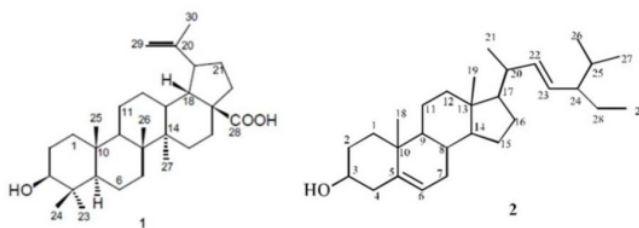


Fig. 3: Structure compounds betulinic acid (1) and stigmasterol (2)

Anticholesterol test using the liebermann-burchard method

The anticholesterol activity was determined by reacting cholesterol with the Liebermann-Burchard reagent. Simvastatin was used as a positive control in this experiment. Measurements were made at

λ_{max} 420 nm. The anticholesterol activity was determined based on the amount of residual cholesterol and calculated based on the percentage inhibition cholesterol. The higher the sample concentration, the smaller the absorbance value and a lower amount of cholesterol residue indicate higher anticholesterol activity.

Table 3: Percentage inhibition (%) cholesterol

Concentration ($\mu\text{g/ml}$)	% Inhibition		
	Betulinic acid (1)	Stigmasterol (2)	Simvastatin
100	71.9 \pm 3.9	24.7 \pm 3.4	86.8 \pm 0.3
75	63.2 \pm 0.9	20.0 \pm 5.4	72.1 \pm 0.1
50	50.71 \pm 1.2	15.3 \pm 1.5	57.6 \pm 2.3
40	41.2 \pm 3.0	12.7 \pm 2.5	54.6 \pm 0.1
30	29.9 \pm 2.5	11.3 \pm 3.5	51.2 \pm 0.2
20	12.1 \pm 3.4	9.3 \pm 2.7	48.3 \pm 0.2
10	7.84 \pm 1.7	5.7 \pm 2.2	47.6 \pm 0.2

Note: The data was given as mean \pm SD; n=3

Table 4: The anticholesterol activity of the compounds

Compounds	IC ₅₀ ($\mu\text{g/ml}$)
Betulinic acid (1)	60.53
Stigmasterol (2)	222.32
Simvastatin	24.68

Based on the absorbance value, the percent inhibition value can be calculated (table 3). A higher percent inhibition value indicates a higher activity. At the same concentrations, the compounds that were isolated showed a lower percent inhibition value than simvastatin. Percent inhibition of the stigmasterol (2) is significantly lower than that of betulinic acid (1). The IC₅₀ values for the compounds and the positive control simvastatin were determined using a regression equation of the relationship between concentration (x) and percentage inhibition cholesterol (y). The IC₅₀ value was inversely proportional to the anticholesterol activity. The smaller the IC₅₀ value, the higher the anticholesterol activity. Based on the linear regression equation, the IC₅₀ value for betulinic acid (1) was 60.53 $\mu\text{g/ml}$, and stigmasterol (2) 222.32 $\mu\text{g/ml}$, while positive control simvastatin 24.68 $\mu\text{g/ml}$ (table 4). The anticholesterol activity of stigmasterol (2) is deficient compared betulinic acid (1), while betulinic acid (1) active anticholesterol but lower anticholesterol activity than simvastatin.

Several other triterpenoid compounds have been reported to have anticholesterol properties. The triterpenoid compounds, 3 β -hydroxy-olean-12-en-28-at acid and 3,19-dihydroxy-urs-12-en-28-at acid found in the *Saurauia vulcani* Korth, showed anticholesterol activity [15]. *Kedaung* (*Parkia timoriana* (DC) Merr) was also found to contain two phytosterol compounds, β -sitosterol and campesterol, which can reduce total blood cholesterol and LDL levels 30–50% by inhibiting cholesterol absorption in the intestinal lumen [25].

CONCLUSION

In this study, the triterpenes betulinic acid (1) and the steroid stigmasterol (2) were isolated from the leaves of *P. canescens*. Betulinic acid (1) had an anticholesterol activity with IC₅₀ of 60.53

$\mu\text{g/ml}$. This is the first report on the isolation of betulinic acid from *P. canescens* and the identification of its anticholesterol activity. The stigmasterol (2) has previously been reported from *P. canescens*

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declared no conflict of interest

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