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Isolation and Characterization of Cholest-5-en-3-ol Compound from the Ethyl Acetate Extract of Stem Bark Ulin Plant (*Eusideroxylon zwageri* Teijm & Binn)

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Abstract: Cholest-5-en-3-ol is an organic compound that belongs to the category of steroidal secondary metabolites. The compound has a double bond and a hydroxyl group and is commonly found in plants that contain oils such as coconut, nuts, and others. So far, there have been no reports of isolating the compound cholest-5-en-3-ol from the extract of the stem bark of the ulin plant. This compound has been found in very small amounts. A maceration method was used to obtain the extract. The isolation method used for the isolation of the compound was chromatography, while for the elucidation of the structure of the compound, UV-Vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR) were used. The ethyl acetate extract is the most abundant with 85.5 g. The isolation results were obtained as a white needle-like solid. Based on the spectra of UV-Vis spectrophotometry, FTIR spectra, and chemical shifts of NMR, the elucidated compound is the cholest-5-en-3-ol.

Keywords: Isolation, Characterization, Ulin, Cholest-5-en-3-ol.

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1. INTRODUCTION

Ulin is one of the indigenous plants of Borneo Island used by the Uud Danum Dayak people living around the Ambalau River. The Uud Danum Dayak tribe often uses ulin leaves to treat fevers, tonics, stomach aches, allergies, and postnatal care (1). Ulin is a plant that is widely used in traditional medicine. A decoction of the bark is used by the people of Kutai and surrounding areas to treat diabetes, toothache, stomach ache, fever, and gynecological problems. In addition to using the ulin plant for furniture, the people of Jambi traditionally use ironwood as a skin medicine (2,3). The bark leaves, and seeds of the ulin plant can be used to treat a variety of ailments. The bark is commonly used for toothache, jaundice, herbal postpartum treatment, and black hair treatment. The leaves and roots can be used for postnatal care, and the seeds can be used for treating black hair (4).

Previously, the presence of secondary metabolites in ulin plants has been reported by LC-MS and GC-MS analysis, including N-cis-feruloyl typamine, 3'-O-methylviolanone, 6-hydroxy-2-[2-(4'-methoxyphe-nyl)ethyl] chromone, B-asarone, and eusiderin A (4,5). The isolation of compounds from the methanolic extract of the stem bark has been reported previously. As for the five isolated compounds contained in the bark of ulin plant, namely two neolignan-derived compounds of benzodioxane type, one bicyclo (3,2,1) octanoid neolignan compound, one aporphine alkaloid-derived compound, and one phenanthrene alkaloid-derived compound isolated from the methanol extract (6). The stem bark of the ulin plant was extracted using hexane and methanol, but no steroid compounds were successfully isolated. Therefore, an attempt was made to isolate steroid compounds from the ethyl acetate extract of the ulin plant stem bark.

Steroidal compounds have not been previously reported in ulin plants. Some steroid compounds have potential health benefits, such as preventing cancer (anti-carcinogenic), acting as antioxidants, and treating conditions such as hypoglycemia, allergies,

asthma, rheumatism, and inflammation. Therefore, the isolation of steroid compounds is carried out, especially the cholest-5-en-3-ol compound.

2. EXPERIMENTAL

2.1. Material

Hexane, ethyl acetate, methanol, acetone, dichloromethane, hydrochloric acid, chloroform, distilled water, Liebermann-Burchard reagent, silica gel, and a sample of the stem bark of the ulin plant collected in the village of Selat, Jambi Province, Indonesia, and identified in the Herbarium of Andalas University (ANDA, Number 311/K-ID/ANDA/ IX/2020).

Table 1: Characteristics of ulin plant.

Characteristics	Name
Kingdom	Plantae
Divisi	Spermatophyta
Sub Divisi	Angiospermae
Kelas	Dicotiledoneae
Ordo	Laurales
Family	Lauraceae
Genus	Eusideroxylon
Species	*Eusideroxylon zwageri* Teijsm. & Binn

2.2. Apparatus

Glassware, separating funnel, dark-colored bottles, aluminum foil, analytical balance (KERNABJ220-4NM), test tube rack, dropping pipette, micro pipette, white Whatman filter paper, grinder, water bath, centrifuge, rotary evaporator (Heidolph Laborota 4000), TLC plate, chromatographic column, UV-Vis Spectrophotometer (Thermo Scientific, Genesys 20), FTIR (UATR FTIR PerkinElmer Frontier C90704 Spectrum IR Version 10.6.1), NMR (Bruker-Avance Neo 500 MHz).

2.3. Extraction

A dried sample of up to 5 kg was taken from Selat village, Bulian Regency, Jambi Province. It was macerated in stages with various solvents, starting with n-hexane, ethyl acetate, and methanol.

2.4. Identification of Steroids in Ethyl Acetate Extract

The ethyl acetate extract is dissolved in a mixture of chloroform and water (1:1 v/v) until a layer is formed. The bottom layer was dripped onto the drip plate, and Liebermann-Burchard reagent was added.

2.5. Isolation of Steroidal Compounds

Isolation begins with thin-layer chromatography (TLC), which is an initial screening to determine the type of eluent to be used for isolation by column chromatography. A small amount of ulin extract is dissolved in the solvent used, then the sample is spotted onto the TLC plate and placed in a vessel saturated with the eluent. The TLC plate is left in the vessel until the eluent reaches a predetermined limit mark. The TLC plate is then removed from the vessel, air dried, and then irradiated under an ultraviolet lamp at a wavelength of 254 nm and 365 nm to make the spots on the TLC plate more visible.

A sample of 60 g was isolated by column chromatography using the SGP system. The eluate collected in a vial (10 mL) was examined for the color pattern on TLC. The same color pattern was combined to obtain several fractions. The fraction with a simple color pattern was tested for purity and melting point using a melting point apparatus.

2.6. Characterization of Steroidal Compounds

The compounds obtained were analyzed with UV-Vis, FTIR, ^{1}HNMR, ^{13}CNMR, HMQC, HMBC, and DEPT.

3. RESULTS AND DISCUSSION

3.1. Extraction

The yield of n-hexane extract was 60 g (1.2% yield), 85.5 g of ethyl acetate extract (1.7% yield), and 121.4 g of methanolic extract (3.2% yield).

3.2. Identification of Steroids in Ethyl Acetate Extract

Identification results using the Libermann-Burchard reagent produced a green color on the drop plate, indicating the presence of steroidal compounds in the ethyl acetate extract.

3.3. Isolation of Steroidal Compound

The isolation procedure is shown in Figure 1. Separation of 60 g of ethyl acetate extract of ulin stem bark using gravity column chromatography with a silica gel stationary phase with a solvent gradient polarity (SGP) eluent system. The falling eluate was collected in 100 mL vials. The eluates were analyzed by TLC at 5 intervals, and those with the same color pattern were combined. After combining, four fractions (A-D fraction) were obtained.

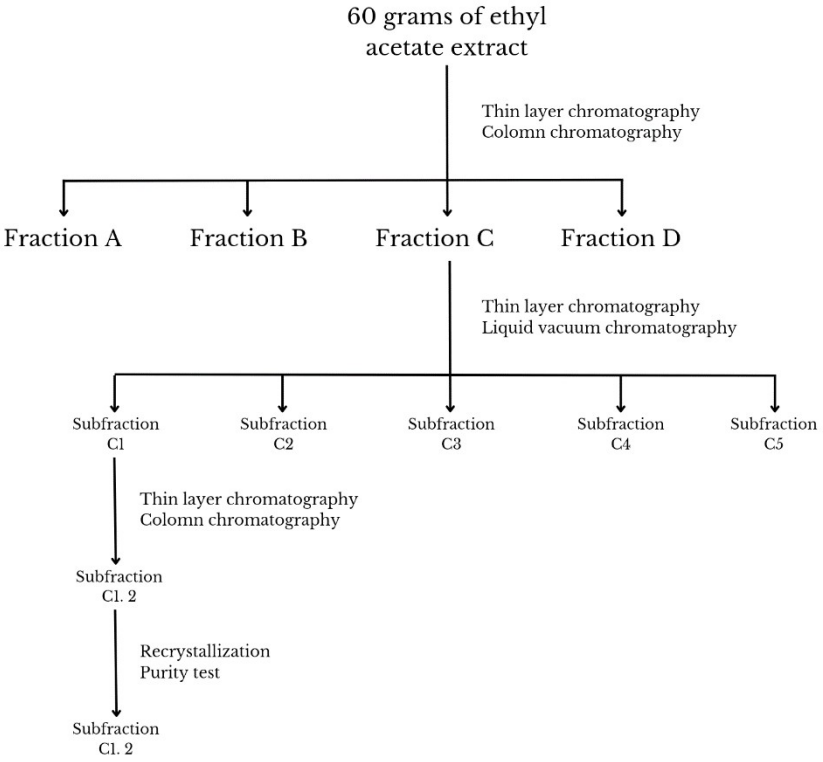

Figure 1: Schematic of the isolation of steroidal compound.

Fraction C was purified using liquid vacuum chromatography with a maximum of 10 grams due to its simpler stain pattern compared to the other fractions. The eluate was collected in a 100 mL vial and separated into 5 subfractions (subfractions C1-C5) using a TLC plate with the same stain pattern. Additionally, the C1 subfraction of 0.787 grams was subjected to column chromatography for further purification.

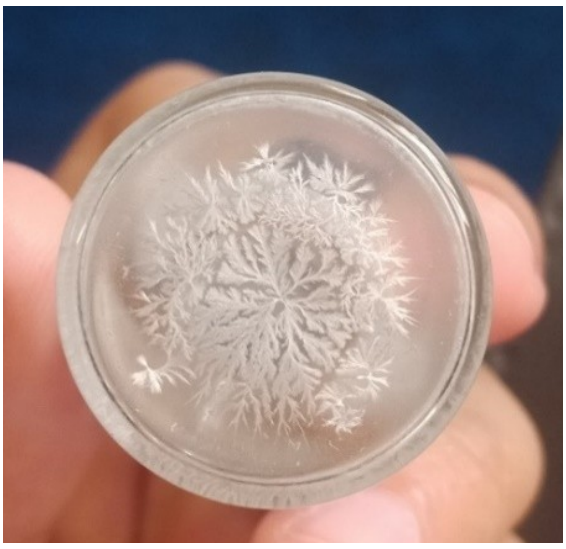

Figure 2: Needle-like solid obtained.

Subfraction C1 was selected due to its simple stain pattern compared to other subfractions, and the fact that the eluate was collected in a 10 mL vial. The same eluate was combined into sub-subfractions (C1.1-C1.9). In sub-subfraction C1.2, a white needle-like solid was observed (Figure 2), which led to further purification of the C1.2 sub-fraction through recrystallization.

The solids tested using TLC with an eluent of hexane and ethyl acetate in a ratio of 8.5:1.5 showed a stain pattern that appeared as a single bluish-green color after being sprayed with Lieberman-Burchard reagent and heated (refer to Figure 3) (7). The compound obtained was tested for its melting point, which was found to be 148-150 °C. Therefore, it can be concluded that the compounds isolated are a group of steroid secondary metabolites (8,9).

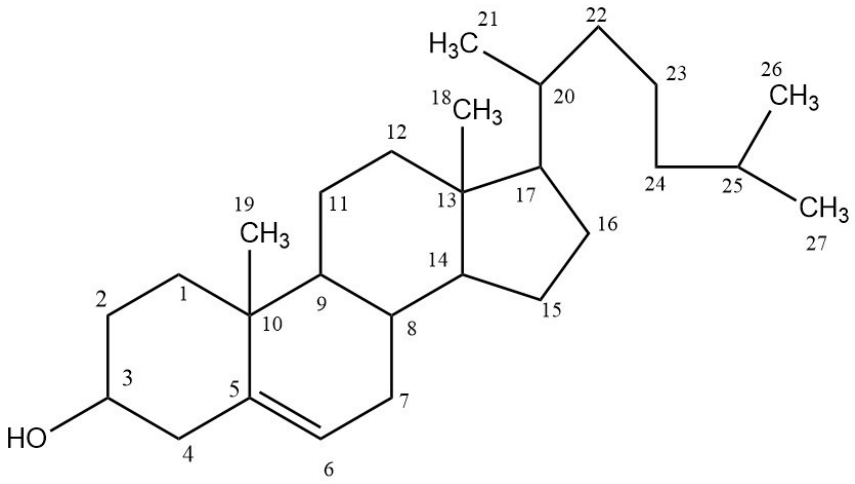

Figure 3: The structure of the cholest-5-en-3-ol.

3.4. Compound Characterization

White powder, UV-Vis λ 207 nm. IR (KBr) v max 3361.20, 2932.94, 2862.79, 1669.38, 1455.35, 1042,05 cm^{-1}; ^{1}H-NMR (d6, 500 MHz, δ, ppm) δ_H 1.828 (H-1, d, *J*=1.48, 2H), 1.750 (H-2, t, *J* = 2.15, 2H), 3.364 (H-3, d, *J* = 0,88), 2.181 (H-4, t, *J* = 2.41 and 1,48, not observed), 5.282 (H-6, d, *J* = 1.00), 1.918 (H-7, dd, *J* = 1.48 and dd, *J* = 1.40, not observed), 1.953 (H-8, q, *J* = 1,37), 0.927 (H-9, d, *J* = 0.61), 1.534 (H-11, s, *J* = 1.38, 2H), 1.196 (H-12, t, *J* = 2.46 and 1.62, not observed), 1.114 (H-14, s, *J* = 1.62), 1.559 (H-15, t, *J* = 1.62 and 0.61, not observed), 1.848 (H-16, t, *J* = 2.15, 2H), 1.236 (H-17, s, *J* = 1.62), 0.693 (H-18, s, *J* = 2.64), 0.939 (H-19, d, *J* = 3.50), 1.171 (H-20, dd, *J* = 1.37), 0.840 (H-21, dd, *J* = 3.69), 2.292 (H-22, s, *J* =2.41, 2H), 1.274 (H-23, t, *J* = 2.46 and 1.38, not observed), 1.188 (H-24, dd, *J* = 2.57, 2H) 1.953 (H-25, dd, *J* = 1.48), 0.810 (H-26, dd, *J* = 3.38), 0.694 (H-27, s, *J* = 2.64); ^{13}C-NMR (d6, 500 MHz) δ_c 37.407 (C-1) 31.667 (C-2) 70.878 (C-3) 42.504 (C-4) 141.524 (C-5) 120.743 (C-6) 31.801 (C-7) 31.945 (C-8) 50.375 (C-9) 36.485 (C-10) 20.964 (C-11) 39.835 (C-12) 42.254 (C-13) 56.825 (C-14) 24.122 (C-15) 28.144 (C-16) 55.980 (C-17) 11.452 (C-18) 19.275 (C-19) 36.111 (C-20) 18.977 (C-21) 33.855 (C-22) 22.909 (C-23) 39.720 (C-24) 31.878 (C-25) 20.628 (C-26) 20.859 (C-27).

3.5. UV-Vis

UV-Vis spectrophotometry was performed in the range of 190 – 500 nm. The measurements revealed a strong absorption peak at a wavelength of 207 nm, indicating the presence of a non-conjugated double bond (C=C) due to the π → π* electronic transition. This was further confirmed by a thin layer chromatography test, where the eluted TLC plate did not show any fluorescent stains upon irradiation with a UV356 nm lamp (10,11).

3.6. FTIR

The FTIR spectrum of the isolated compound displays absorption at the wavenumber 3361.20 cm^{-1}, indicating the presence of hydroxyl groups (-OH). Technical abbreviations have been explained upon first use. This is confirmed by the absorption wave number of 1042.05 cm^{-1}, which indicates the presence of the C-O group. The strain between oxygen and hydrogen is also evident. In the absorption spectrum, wave numbers 2932.94 cm^{-1} and 2862.79 cm^{-1} indicate the presence of primary and secondary aliphatic C-H groups, as evidenced by the symmetric stretching. The bending at wave number 1455.35 cm^{-1} is the area for C-H alkane groups. The absorption at wave number 1669.38 cm^{-1} indicates the presence of an unconjugated C=C double bond, while there is no absorption at a wavelength of 1500 cm^{-1}. Based on the spectrum results, it was concluded that the isolated compounds are steroid secondary metabolites. This is supported by the presence of hydroxy groups (-OH) and unconjugated double bonds (10,12).

3.7. NMR

The Nuclear Magnetic Resonance (NMR) used in analyzing the structure of the isolated results, namely ^{13}C-NMR, aims to determine the number of carbon atoms and their types in compounds isolated from ^{1}H-NMR, which aims to determine the number of protons or hydrogen atoms and the type of chemical environment.

The compound was analyzed using ^{13}C-NMR (100 MHz) and DEPT 135, revealing the presence of 27 carbon atoms. A carbon signal was observed at 70.878 ppm (C3) in the ^{13}C-NMR spectrum, which is suspected to be bonded to a hydroxyl group (-OH). This was supported by FTIR spectral data, which showed an absorption wave number of 3361.20 cm^{-1}, indicating the presence of a hydroxyl functional group (-OH). The IR spectrum shows an absorption wavenumber of 1669.38 cm^{-1}, indicating the presence of a double bond. This is supported by the carbon signals at chemical shifts of 120.743 ppm (C-6) and 141.524 ppm (C-5). The chemical shifts at ^{13}C-NMR and ^{1}H-NMR can be compared with those found in the literature (Table 2) (10,13).

Table 2: ^{1}H-NMR (500 MHz, d6) and ^{13}C-NMR (500 MHz, d6) isolated compounds and cholest-5-en-3-ol comparatory literature data.

Number of Carbon	DEPT 135	^{13}C-NMR isolated compounds (ppm)	^{1}H-NMR isolated compounds (ppm)	^{13}C-NMR Literature (ppm)	^{1}H-NMR Literature (ppm)
C1	CH_2	37.407	1.828 (2H)	37.2	
C2	CH_2	31.667	1.750 (2H)	31.6	
C3	CH	70.878	3.364	71.8	3.524
C4	CH_2	42.504	2.181 (not observed)	42.3	2,280
C5	C	141.524	-	140.6	
C6	CH	120.743	5.282	121.4	5.349
C7	CH_2	31.801	1.918 (not observed)	31.9	
C8	CH	31.878	1.953	31.9	
C9	CH	50.375	0.927	50.2	
C10	C	36.485	-	36.5	
C11	CH_2	20.964	1.534 (2H)	21.1	
C12	CH_2	39.835	1.196 (not observed)	39.8	
C13	C	42.254	-	42.3	
C14	CH	56.825	1.114	56.8	
C15	CH_2	24.122	1.559 (not observed)	24.3	
C16	CH_2	28.144	1.848 (2H)	28.3	
C17	CH	55.980	1.236	56.1	
C18	CH_3	11.404	0.693	11.9	0.677
C19	CH_3	19.275	0.939	19.4	1.006
C20	CH	36.111	1.171	35.8	
C21	CH_3	18.977	0.840	18.7	0.915
C22	CH_2	33.855	2.292 (2H)	36.2	
C23	CH_2	22.903	1.274 (not observed)	23.8	
C24	CH_2	39.720	1.188 (2H)	39.5	
C25	CH	31.878	1.953	28.0	
C26	CH_3	20.628	0.810	22.6	0.867
C27	CH_3	20.859	0.694	22.8	0.862

Based on Table 1, the isolated compound is cholest-5-en-3-ol, as evidenced by the chemical shifts of ^{13}C-NMR and ^{1}H-NMR, which are similar to the shifts of ^{13}C-NMR and ^{1}H-NMR of the compound in the literature.

4. CONCLUSION

The compound cholest-5-en-3-ol was isolated from ulin plants using maceration and chromatography methods. The ulin plant stem bark was the initial source of this compound. After maceration, three extracts were obtained: methanol, ethyl acetate, and methanol. Ethyl acetate extracts were chosen for further isolation as they had not been previously isolated. The isolation product was a white needle-like solid. The compounds were characterized using UV-Vis, FTIR, ^{1}HNMR, ^{13}CNMR, and DEPT to determine the number and type of protons and carbons and the position of the compound structure.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

6. ACKNOWLEDGMENTS

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