

## THREE FRIEDELANE TRITERPENOIDS FROM THE BARK OF *SALIX ALBA* L. (WHITE WILLOW)

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### Abstract

White willow (*Salix alba* L.) grows throughout Africa, North America, Europe, and Asia. The genus *Salix* has traditionally been used in folk medicine for different kinds of pain due to the presence of salicylic acid. This paper highlights the isolation of three friedelane triterpenoids: 3-oxo friedelane (L-1, 0.006 %, m.pt 258-260 °C), 3-β-hydroxyl friedelane (L-2, 0.04 %, m.pt 275-277 °C) and 3-oxofriedelan-21-acetate (L-3, 0.046 %, m.pt 303-305 °C) from the pet ether extract of the bark of *Salix alba* L. These compounds were isolated using column chromatographic method and identified by FT IR, <sup>1</sup>H NMR, and <sup>13</sup>CNMR,

**Keywords:** *Salix alba* L., 3-oxo friedelane, 3-β-hydroxyl friedelane, 3-oxofriedelan-21-acetate

### Introduction

Terpenes are the most extensive and varied class of secondary metabolites produced by plants. Numerous scholars have detailed the wide uses of terpenoids (derivatives of terpene containing different functional groups) for the treatment of many diseases due to their broad range of biological activities. More than 400 naturally occurring friedelane triterpenoids have been identified. Friedelin and 3β-friedelinol are pentacyclic triterpenoids commonly distributed in plants and are found in edible fruits, vegetables and frequently coexist with each other. Friedelin and its derivative 3β-friedelinol are reported to have significant pharmacological potential, including antibacterial, anti-viral, and cytotoxic properties. Friedelane triterpenoids could be considered as promising candidates in drug development against human coronaviruses, including SARS-CoV-2 (Radi, *et al.*, 2023). The aim of this paper is structural identification, physicochemical properties and spectral data of the isolated organic constituents from the bark extract of *Salix alba* L. The bark of *Salix alba* L. is particularly used to treat many different types of pain including rheumatic pain, back pain, toothache and menstrual cramps. *Salix alba* L., commonly known as white willow, is the original source of salicin. It belongs to Salicaceae, and its Myanmar name is Moe-ma-kha.

The genus *Salix* have been reported to possess altogether 322 secondary metabolites including flavonoids, phenolic glycosides, organic acids, non-phenolic glycosides, sterols and terpenes, lignins, and non volatile fatty acids (Tawfeek *et al.*, 2021).



**Figure 1.** Photographs of plant of *Salix alba* L. and stem bark

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## Materials and Methods

### Sample Collection and Preparation

The bark of *Salix alba* L. was collected from Yankin township, Yangon Region, in April, 2016. The scientific name of this plant was verified at the Department of Botany, Dagon University. The sample was washed thoroughly with water and air dried at the room temperature. The dried samples were cut into small pieces and ground into fine powder by using a grinder.

### Chemicals and Reagents

All the solvents and chemical reagents were used of the laboratory grade.

### Apparatus and Instruments

Gallenkamp melting point apparatus, FT IR (8400) spectrophotometer (Shimadzu, Japan), and NMR spectrometer (JEOL, USA) with 500 MHz for  $^1\text{H}$  NMR spectra and 125 MHz for  $^{13}\text{C}$  NMR spectra were used. The NMR spectra were run on the  $\text{CDCl}_3$  solution of the sample.

### Isolation of Friedelane Triterpenoids from the Bark of *Salix alba* L.

Three compounds; L-1, L-2 and L-3 (friedelane triterpenoids) were isolated from the petroleum ether extract of the dried powder of bark of *Salix alba* by column chromatographic method. Firstly, the dried powder (300 g) *Salix alba* bark was directly soaked with 1 L of petroleum ether (PE) (60- 80 °C) at room temperature, for one week and filtered. This procedure was repeated three times. The combined filtrates were evaporated under reduced pressure by means of a rotatory evaporator. The petroleum ether (1.27 g) extract were fractionated on a glass column (60 cm  $\times$  1.5 cm) packed with 150 g of silica gel (40-60  $\mu\text{m}$ , Merck). Elution was carried out by 100% petroleum ether, followed by gradient elution with petroleum ether and ethyl acetate (EA) mixtures; 90:1 to 40:1 v/v. The flow rate was adjusted to about one drop per five seconds. Six main fractions F-I, F-II, F-III, F-IV, F-V, and F-VI were collected after combination of different fractions on the basis of their behaviors on TLC. The isolated compound L-1 (20 mg, 0.006%, as colourless needle shape crystals) was purified from fraction F-II by washing with pet ether and followed by crystallization with chloroform. The compound L-2 (120 mg, 0.04%, colourless powder) was obtained by rechromatography of fraction F-IV, on a silica gel column (60 x 0.75 cm), using 30 g of silica gel and gradient elutions with 100% petroleum ether and petroleum ether: ethyl acetate (80:0.1 to 80:1 v/v) mixtures. Compound L-3 (138 mg, 0.046%) in the form of needle shaped crystals, was obtained through rechromatography of fraction F-VI, using silica gel column and gradient elution with 100% petroleum ether, petroleum ether:ethyl acetate (90:0.1 to 40:1 v/v) mixtures. All the isolated compounds; L-1, L-2 and L-3 were treated with colour reaction tests and recorded the  $R_f$  values. The melting point of each isolated compound was measured using Gallenkamp melting point apparatus. FT IR and NMR spectra were recorded on FT IR (8400) and NMR (JEOL, USA) spectrometers.

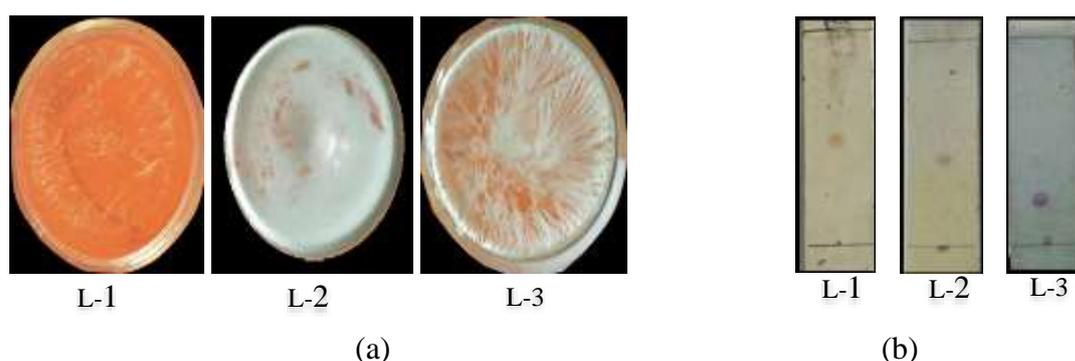
## Results And Discussion

### Physicochemical Characterization and Identification of Isolated Compounds

All the isolated compounds; L-1, L-2 and L-3 were UV inactive under UV lamp (254 nm and 365 nm) and the type of compounds were preliminary checked by colour reaction tests as well as their  $R_f$  values ( 0.48, 0.33 and 0.28) with PE: EA (15: 1 v/v) eluent as presented in Table 1 and Figure 2. These compounds were soluble in petroleum ether, chloroform, ethyl acetate, and

methanol but insoluble in water. The melting points of L-1, L-2 and L-3 were found to be 258-260 °C, 275 -277 °C and 303- 305 °C, respectively.

Compound L-1: The FT IR spectrum of compound L-1 indicated the strong absorption band of carbonyl functional group at  $1713\text{ cm}^{-1}$  which appeared due to the C=O stretching vibration of carbonyl group of cyclohexane. The absorption bands at  $2917\text{ cm}^{-1}$  and  $2848\text{ cm}^{-1}$  were assigned to symmetric and asymmetric C-H stretching in  $-\text{CH}_2$  and  $-\text{CH}_3$  groups. The absorption bands at  $1461\text{ cm}^{-1}$  and  $1388\text{ cm}^{-1}$  correspond to the bending vibration of  $-\text{CH}_2$  and  $-\text{CH}_3$  groups. The absorption band at  $719\text{ cm}^{-1}$  corresponds to rocking of  $-\text{CH}_2$  group. In  $^1\text{H}$  NMR spectra of compound L-1 indicated that it contained totally 50 protons. It provided eight methyl proton signals at  $\delta_{\text{H}}$  0.87 ppm (H-23, d,  $J$  6.4 Hz), 0.72 ppm (H-24, s), 0.88 ppm (H-25, s), 1.00 ppm (H-26, s), 1.05 ppm (H-27, s), 1.18 ppm (H-28, s), 1.00 ppm (H-29, s) and 0.95 ppm (H-30, s). In addition, the numerous methylene signals appeared at  $\delta_{\text{H}}$  1.23 ppm to 2.41 ppm.



**Figure 2.** (a) Photograph of crystals, and (b) thin layer chromatograms of isolated compounds L-1, L-2 and L-3 (PE: EA, 15:1 v/v, sprayed with 5%  $\text{H}_2\text{SO}_4$ ,  $\Delta$ )

**Table 1. Results of Physicochemical Properties of the Isolated Compounds**

Reagent	Observation			Remark
	L-1	L-2	L-3	
5 % $\text{H}_2\text{SO}_4$ , $\Delta$	Brown colour	Brown colour	Brown colour	Presence of organic compound
10 % $\text{FeCl}_3$	No colour change	No colour change	No colour change	Absence of phenolic OH
Bromocresol green	No colour change	No colour change	No colour change	Absence of organic acid
Liebermann-Burchard, $\Delta$	Light pink colour	Light pink colour	Pale pink colour	Terpenoid compound
Anisaldehyde/ $\text{H}_2\text{SO}_4$ , $\Delta$	Pale violet colour	Violet colour	Purple colour	Terpenoid compound
$R_f$ , (PE:EA 15:1 v/v)	0.48	0.33	0.28	

\*Randerath, 1966

The  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) of compound L-1 indicated the presence of eight methyl carbons, eleven methylene carbons, four methine carbons and six quaternary carbons and one carbonyl carbon. The carbon signals for eight methyl carbons were observed at  $\delta_{\text{C}}$  7.1 ppm (C-23), 15.0 ppm (C-24), 18.3 ppm (C-25), 20.6 ppm (C-26), 19.0 ppm (C-27), 32.4 ppm (C-28), 35.4 ppm (C-29) and 32.1 ppm (C-30) whereas eleven methylene carbons were assigned at  $\delta_{\text{C}}$  22.6 ppm (C-1), 41.9 ppm (C-2), 41.7 ppm (C-6), 18.6 ppm (C-7), 36.0 ppm (C-11), 30.9 ppm

(C-12), 32.8 ppm (C-15), 36.4 ppm (C-16), 35.7 ppm (C-19), 33.1 ppm (C-21) and 39.6 ppm (C-22). And also four methine carbons appeared at  $\delta_c$  58.6 ppm (C-4), 53.5 ppm (C-8), 59.9 ppm (C-10) and 43.2 ppm (C-18). Moreover, six quaternary carbons appeared at  $\delta_c$  42.5 ppm (C-5), 37.8 ppm (C-9), 40.1 ppm (C-13), 38.7 ppm (C-14), 30.4 ppm (C-17) and 28.5 ppm (C-20). The one carbonyl group was found as  $\delta_c$  213.4 ppm (C-3). According to the results, the molecular formula of compound L-1 is C<sub>30</sub>H<sub>50</sub>O.

**Table 2. NMR Spectral Data of Isolated Compound L-1 with 3-oxo Friedelane (Friedelin)**

C Position	C-Type	Observed ( $\delta$ in ppm, CDCl <sub>3</sub> )		3-oxo Friedelane * ( $\delta$ in ppm, CDCl <sub>3</sub> )	
		<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
		(500 MHz)	(125 MHz)	(400 MHz)	(100MHz)
1	CH <sub>2</sub>	1.97	22.6	1.97,1.57	22.3
2	CH <sub>2</sub>		41.9	2.30, 2.29	41.5
3	C=O		213.4		213.3
4	CH	2.25(m)	58.6	2.25	58.3
5	C		42.5		42.2
6	CH <sub>2</sub>		41.7	1.74	41.3
7	CH <sub>2</sub>		18.6	1.38	18.3
8	CH		53.5		53.1
9	C		37.8	1.53	37.5
10	CH		59.9	1.47	59.5
11	CH <sub>2</sub>		36.0		35.6
12	CH <sub>2</sub>		30.9		30.5
13	C		40.1		39.7
14	C		38.7		38.3
15	CH <sub>2</sub>		32.8	1.59	32.5
16	CH <sub>2</sub>		36.4		36.0
17	C		30.4		30.0
18	CH		43.2		42.9
19	CH <sub>2</sub>		35.7		35.4
20	C		28.5		28.2
21	CH <sub>2</sub>		33.1	1.53,1.38	32.3
22	CH <sub>2</sub>		39.6	0.94	39.3
23	CH <sub>3</sub>	0.87 ( <i>d</i> , <i>J</i> =6.4 Hz)	7.1	0.87( <i>d</i> )	6.9
24	CH <sub>3</sub>	0.72( <i>s</i> )	15.0	0.71( <i>s</i> )	14.2
25	CH <sub>3</sub>	0.88( <i>s</i> )	18.3	0.86( <i>s</i> )	18.1
26	CH <sub>3</sub>	1.00( <i>s</i> )	20.6	1.06( <i>s</i> )	20.3
27	CH <sub>3</sub>	1.05( <i>s</i> )	19.0	1.04( <i>s</i> )	18.7
28	CH <sub>3</sub>	1.18( <i>s</i> )	32.4	1.17( <i>s</i> )	32.1
29	CH <sub>3</sub>	1.00( <i>s</i> )	35.4	0.99( <i>s</i> )	35.1
30	CH <sub>3</sub>	0.95( <i>s</i> )	32.1	0.94( <i>s</i> )	31.8

\* Odeh, *et al.*, 2016

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectral data of compound L-1 matched with the reported compound 3-oxofriedelane see in Table 2 and Figures 3 and 5 (Odehl, *et al.*, 2016). Quintansa, *et al.*, 2014 reported that melting point of 3-oxofriedelane is 258-260°C. Therefore, compound L-1

was finally proved to be 3-oxofriedelane by comparison of its  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectral data and melting point. The structure of 3-oxofriedelane is shown in Figure 3(a).

Compound L-2: In the FT IR spectrum of compound L-2, the absorption band at  $3466\text{ cm}^{-1}$  corresponds to the O-H stretching and frequencies at  $1448\text{ cm}^{-1}$  and  $1384\text{ cm}^{-1}$  which were indicative of bending O-H. The absorption bands at  $1257\text{ cm}^{-1}$ ,  $1172\text{ cm}^{-1}$ ,  $1020\text{ cm}^{-1}$  and  $1000\text{ cm}^{-1}$  were observed for C-O stretching vibration of alcohol. The absorption bands at  $2930\text{ cm}^{-1}$  and  $2869\text{ cm}^{-1}$  represent the asymmetric and symmetric C-H stretching vibration of  $-\text{CH}_2$  and  $-\text{CH}_3$  groups. Besides, in plane bending of O-H of primary and secondary alcohols which was coupled with C-H bending vibration of  $-\text{CH}_2$  and  $-\text{CH}_3$  group occurred at  $1420\text{--}1330\text{ cm}^{-1}$  (Silverstein, *et al.*, 2009). The  $^1\text{H}$  NMR spectrum indicated eight methyl protons signals at  $\delta_{\text{H}}$  1.01 ppm (H-23, d,  $J = 7.5\text{ Hz}$ ), 1.02 ppm (H-24, s), 0.87 ppm (H-25, s), 0.96 ppm (H-26, s), 0.98 ppm (H-27, s), 1.18 ppm (H-28, s), 0.94 ppm (H-29, s) and 1.02 ppm (H-30, s). In addition, one singlet signal of methine proton attached with one -OH appeared at  $\delta_{\text{H}}$  3.73 ppm.

**Table 3. NMR Spectral Data of Isolated Compound L-2 and 3- $\beta$ -hydroxyl Friedelane (3- $\beta$ - Friedelinol)**

C Position	C-Type	Observed (ppm)		3- $\beta$ -hydroxyl Friedelane* (ppm)	
		$^1\text{H}$ ( $\delta$ ) (500 MHz, $\text{CDCl}_3$ )	$^{13}\text{C}$ ( $\delta$ ) (125MHz, $\text{CDCl}_3$ )	$^1\text{H}$ ( $\delta$ ) (400 MHz, $\text{CDCl}_3$ )	$^{13}\text{C}$ ( $\delta$ ) (100 MHz, $\text{CDCl}_3$ )
1	$\text{CH}_2$		16.2	1.69( <i>dt</i> ,13,3, $\text{H}_{\text{ax}}$ ) 1.45( $\text{H}_{\text{eq}}$ )	16.2
2	$\text{CH}_2$		35.7	1.99( <i>qd</i> ,13,3, $\text{H}_{\text{ax}}$ ) 0.99( $\text{H}_{\text{eq}}$ )	36.1
3	CH-OH	3.73	73.1	3.81( <i>q</i> like,2, $\text{H}_{\text{eq}}$ )	71.6
4	CH		49.6		49.6
5	C		38.2		38.1
6	$\text{CH}_2$		42.1	1.76( <i>td</i> ,12,3, $\text{H}_{\text{eq}}$ ) 0.99( $\text{H}_{\text{ax}}$ )	41.9
7	$\text{CH}_2$		17.9	1.41,1.39	17.7
8	CH		53.6	1.28( $\text{H}_{\text{ax}}$ )	53.3
9	C		37.5	1.25( $\text{H}_{\text{ax}}$ )	37.2
10	CH		61.8	0.93( <i>dd</i> ,12, 2, $\text{H}_{\text{ax}}$ )	61.7
11	$\text{CH}_2$		35.6	1.42, 1.24	35.7
12	$\text{CH}_2$		31.0	1.34, 1.31	30.7
13	C		38.7		38.4
14	C		40.1		39.7
15	$\text{CH}_2$		33.2	1.53, 1.28	32.3
16	$\text{CH}_2$		36.5	1.46, 1.18	35.9
17	C		30.0		30.1
18	CH		43.2	1.56( <i>dd</i> ,11,6, $\text{H}_{\text{ax}}$ )	42.9
19	$\text{CH}_2$		35.9	1.37, 1.21	35.4
20	C		28.7		28.2
21	$\text{CH}_2$		32.7	1.49,1.28	32.9
22	$\text{CH}_2$		39.7	0.93( <i>dd</i> ,12,2, $\text{H}_{\text{ax}}$ ) 1.49( $\text{H}_{\text{eq}}$ )	39.3
23	$\text{CH}_3$	1.01 ( <i>d</i> , $J=7.5\text{ Hz}$ )	12.0	1.02 ( <i>d</i> , $J=7.0\text{ Hz}$ )	12.1

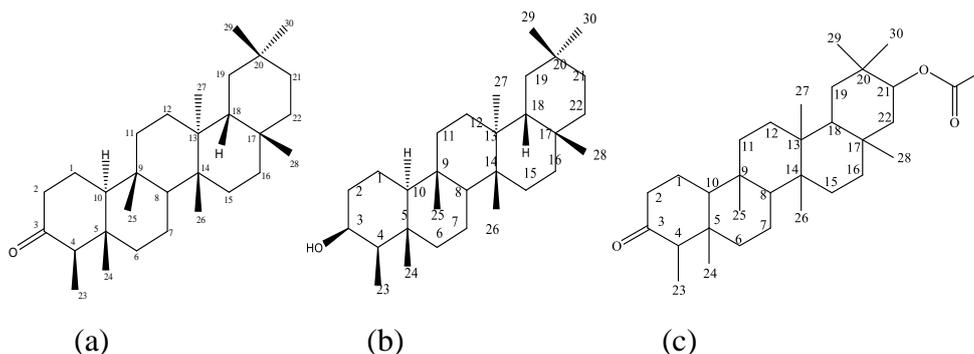
C Position	C-Type	Observed (ppm)		3-β-hydroxyl Friedelane * (ppm)	
		<sup>1</sup> H (δ) (500 MHz, CDCl <sub>3</sub> )	<sup>13</sup> C (δ) (125MHz, CDCl <sub>3</sub> )	<sup>1</sup> H (δ) (400 MHz, CDCl <sub>3</sub> )	<sup>13</sup> C (δ) (100 MHz, CDCl <sub>3</sub> )
24	CH <sub>3</sub>	1.02(s)	16.8	1.10(s)	16.6
25	CH <sub>3</sub>	0.87(s)	18.6	0.89(s)	18.4
26	CH <sub>3</sub>	0.96(s)	19.0	0.99(s)	18.6
27	CH <sub>3</sub>	0.98(s)	20.5	1.02(s)	20.1
28	CH <sub>3</sub>	1.18(s)	32.5	1.18(s)	32.1
29	CH <sub>3</sub>	0.94(s)	35.4	0.97(s)	35.0
30	CH <sub>3</sub>	1.02(s)	32.2	1.02(s)	31.9

\*Salazar, *et al.*, 2000

The <sup>1</sup>H NMR spectrum of compound L-2 indicated totally 52 protons. The <sup>13</sup>C NMR spectrum indicated the presence of eight methyl carbons, eleven methylene carbons, five methine carbons and six quaternary carbons. The carbon signals for eight methyl carbons were observed at δ<sub>c</sub> 12.0 ppm (C-23), 16.8 ppm (C-24), 18.6 ppm (C-25), 19.0 ppm (C-26), 20.5 ppm (C-27), 32.5 ppm (C-28), 35.4 ppm (C-29), 32.2 ppm (C-30) whereas eleven methylene carbons were assigned at δ<sub>c</sub> 16.2 ppm (C-1), 35.7 ppm (C-2), 42.1 ppm (C-6), 17.9 ppm (C-7), 35.6 ppm (C-11), 31.0 ppm (C-12), 33.2 ppm (C-15), 36.5 ppm (C-16), 35.9 ppm (C-19), 32.7 ppm (C-21) and 39.7 ppm (C-22). And also four methine carbons appeared at δ<sub>c</sub> 49.6 ppm (C-4), 53.6 ppm (C-8), 61.8 ppm (C-10) and 43.2 ppm (C-18). Moreover, six quaternary carbons appeared at δ<sub>c</sub> 38.2 ppm (C-5), 37.5 ppm (C-9), 38.7 ppm (C-13), 40.1 ppm (C-14), 30.0 ppm (C-17) and 28.7 ppm (C-20). One hydroxyl group was found that it attached to C-3 at δ<sub>c</sub> 73.1 ppm. The NMR spectral data of the isolated compound L-2 agree with the previously reported NMR spectral data of 3-β-hydroxyl friedelane (friedelinol), Table 3 and Figures 3 and 6 (Salazar, *et al.*, 2000). Thus, compound L-2 is suggested to be 3-β-hydroxyl friedelane, with the molecular formula of C<sub>30</sub>H<sub>52</sub>O, as confirmed by its spectral data and physicochemical properties. Figure 3(b) shows the structure of 3-β-hydroxyl friedelane.

Compound L-3: In the FT IR spectrum of isolated compound L-3, the two strong absorption bands at 1715 cm<sup>-1</sup> and 1726 cm<sup>-1</sup> are due to stretching C=O of carbonyl group of hexacyclic ketone and ester, because the carbonyl group of ester has higher frequency than a normal ketone (Silverstein, *et al.*, 2009). The two strong absorption bands at 1245 and 1027 cm<sup>-1</sup> show the C-O stretching of an ester. The absorption bands at 2923 cm<sup>-1</sup> and 2862 cm<sup>-1</sup> show symmetric and asymmetric C-H stretching vibration of -CH<sub>2</sub> and -CH<sub>3</sub> groups. The absorption band at 1453 cm<sup>-1</sup> and 1388 cm<sup>-1</sup> are due to C-H bending vibrations of -CH<sub>2</sub> and -CH<sub>3</sub> groups. Moreover, <sup>1</sup>H NMR spectral data of compound L-3 revealed the presence of 52 protons. It showed one secondary methyl signal at δ<sub>H</sub> 0.87 ppm (3H, d, J=6.5 Hz, H-23), six tertiary methyl proton signals at 0.71 ppm (3H, s, H-24), 0.86 ppm (3H, s, H-25), 0.94 ppm (3H, s, H-26), 1.07 ppm (3H, s, H-27), 1.27 ppm (3H, s, H-28), 1.09 ppm (3H, s, H-29), 0.94 ppm (3H, s, H-30) and one methyl proton signal at 2.02 ppm (3H, s) of acetate (-OCOCH<sub>3</sub>), the remaining methylene and methine signals showed chemical shifts between 1.27 - 1.95 ppm. A methine proton signal at 4.9 ppm (1H, dd, J =15, 5 Hz) is indicative of an attached acetate group. Its chemical shift and multiplicity suggest that this may correspond to a ring methine carbon. The isolated compound L-3 revealed that it has eight methyl carbons: at δ<sub>c</sub> 6.9 ppm (C-23), 14.6 ppm (C-24), 18.0 ppm (C-25), 18.7 ppm (C-26), 18.9 ppm (C-27), 32.6 ppm (C-28), 30.3 ppm (C-29), and 26.1 ppm

(C-30); one methyl carbon of acetate (-OCOCH<sub>3</sub>) at  $\delta_C$  21.2 ppm; two carbonyl carbons: at  $\delta_C$  213.2 ppm (C-3) for cyclic ketone and 171.3 ppm suggested the presence of another carbonyl carbon of acetate attached at ring carbon; four methine carbons: at  $\delta_C$  58.2 ppm (C-4), 52.2 ppm (C-8), 59.4 ppm (C-10) and 43.3 ppm (C-18); six quaternary carbons: at  $\delta_C$  42.1 ppm (C-5), 37.4 ppm (C-9), 39.2 ppm (C-13), 38.8 ppm (C-14), 31.8 ppm (C-17), and 33.4 ppm (C-20); and ten methylene carbons: at  $\delta_C$  22.2 ppm (C-1), 41.5 ppm (C-2), 41.2 ppm (C-6), 18.2 ppm (C-7), 34.6 ppm (C-11), 30.5 ppm (C-12), 31.5 ppm (C-15), 36.5 ppm (C-16), 35.8 ppm (C-19), and 43.6 ppm (C-22). <sup>13</sup>C NMR spectral data of compound L-3 was presented alongside the NMR spectral data of 3-oxofriedelane (compound L-1) and 3- $\beta$ -hydroxy friedelan-21  $\alpha$ -acetate in Table 4 (Patra and Chaundhuri, 1987 and Anjaneyulu *et al.*, 1993). By comparing <sup>13</sup>C NMR spectral data, the upfield shift of C=O at  $\delta_C$  213.2 ppm may be considered that same chemical shift of 3-position of 3-oxo friedelane. The chemical shift  $\delta_C$  171.3 ppm for C=O of acetate group, it may be attached on ring D or E of pentacyclic triterpenoid of friedelane type that can be seen in Figure 3. The chemical shift 76.4 ppm for methine carbon (C-21) revealed the attachment of acetate. Furthermore, the comparable chemical shifts of L-1 (3-oxo friedelane) and L-3 indicated the downfield shift at  $\delta_C$  33.4 ppm for C-20 and 43.6 ppm for C-22 and upfield shift at 30.3 ppm for C-29 and 26.1 ppm for C-30 see in Table 4, Figures 5 and 7. Moreover, when a ring carbon atom is attached to an acetate group, the presence of carbonyl group induces a  $\beta$ -effect at the  $\beta$ -position carbon, leading to a downfield shift (higher ppm). At the same time, the electron donating methyl group in the acetate induces  $\gamma$ -effect at the  $\gamma$ -position carbon, leading to an upfield shift (lower ppm). In compound L-3, since an acetate group would attach at 21 position that caused  $\beta$ -effect on C-20 (+ 4.9 ppm) and C-22 (+ 4.0 ppm) and  $\gamma$ -effect on C-29 (-5.1 ppm) and C-30 (-6.0 ppm) were comparatively observed with 3-oxo friedelane (L-1) see in Table 4.



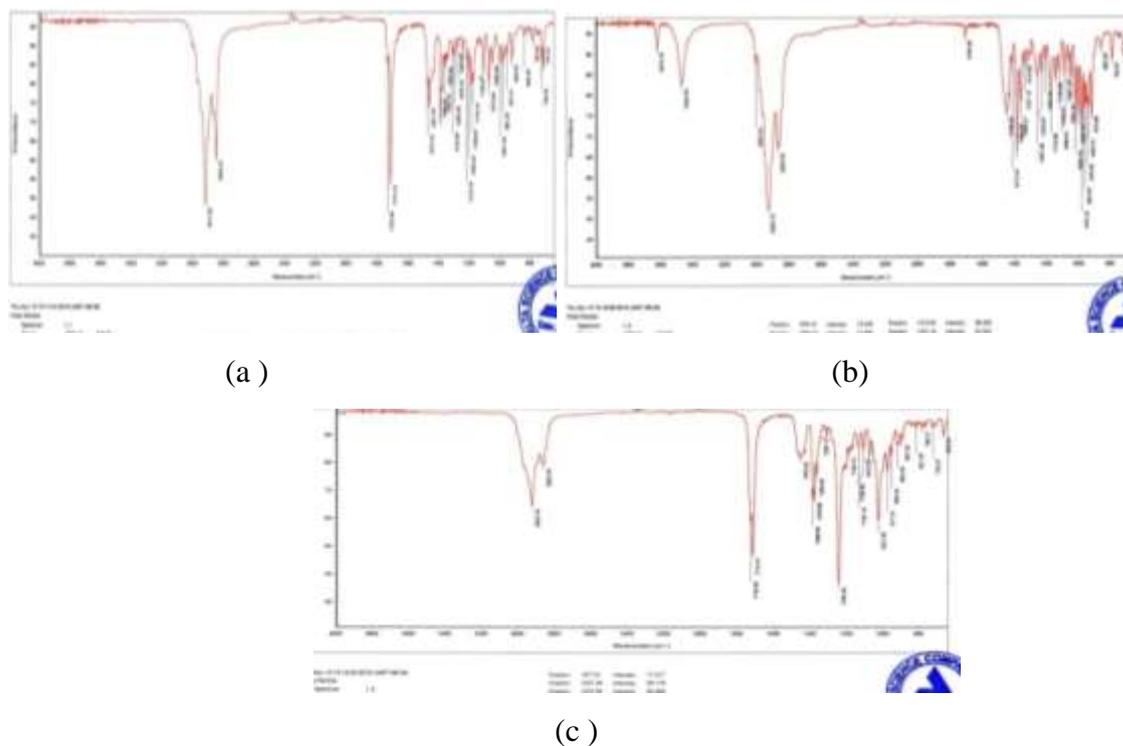
**Figure 3.** The possible chemical structure of isolated compounds  
 (a) 3-oxofriedelane, L-1, (b) 3- $\beta$ -hydroxyl friedelane, L-2 and  
 (c) 3-oxofriedelan-21-acetate. L-3

NMR spectral data of L-3 provided evidence of agreement and consistency with an acetate group attached at 21-position of 3-oxofriedelane. Thus, the possible chemical structure of isolated compound L-3 may be considered as 3-oxofriedelan-21-acetate see in Figure 3(c).

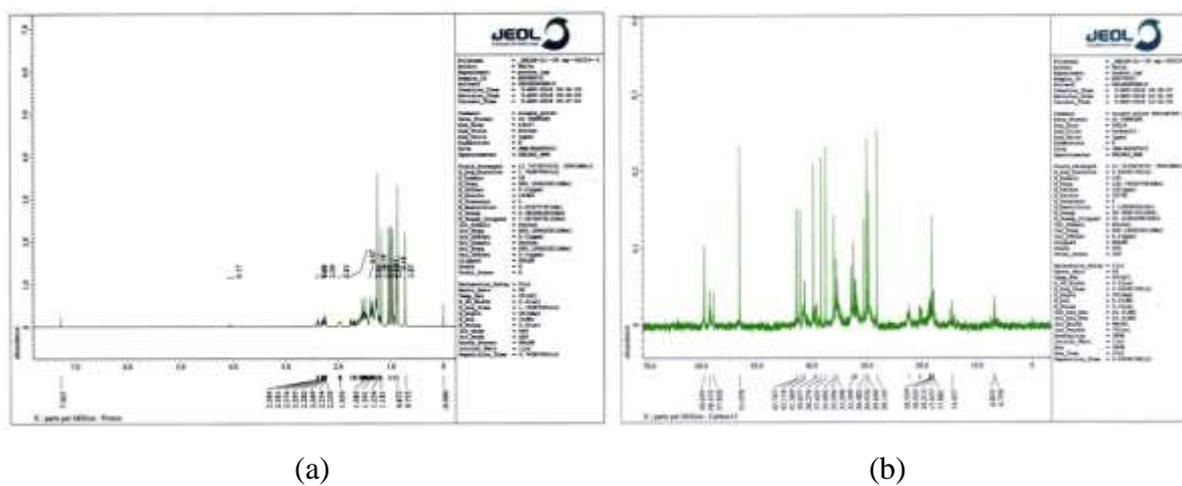
**Table 4. NMR Spectral Data of Isolated Compound L-3 and 3-oxofriedelane and 3 $\beta$ -OH friedelan-21 $\alpha$ - acetate**

Carbon position	Observed data of L-3 ( $\delta$ value ppm, CDCl <sub>3</sub> J value in Hz)		3-oxofriedelane ( $\delta$ value ppm, CDCl <sub>3</sub> )	*3 $\beta$ -OH friedelan-21 $\alpha$ - acetate ( <sup>13</sup> CNMR, $\delta$ value ppm, CDCl <sub>3</sub> , 50.32 MHz)
	<sup>1</sup> H NMR, 500MHz	<sup>13</sup> CNMR, 125MHz	<sup>13</sup> CNMR, 125MHz	
1		22.2	22.6	15.7
2		41.5	41.9	34.9
3		213.2	213.4	72.5
4		58.2	58.6	49.0
5		42.1	42.5	37.7
6		41.2	41.7	41.6
7		18.2	18.6	17.4
8		52.2	53.5	52.2
9		37.4	37.8	37.0
10		59.4	59.9	61.2
11		34.6	36.0	35.2
12		30.5	30.9	30.3
13		39.2	40.1	39.1
14		38.8	38.7	38.5
15		31.5	32.8	31.0
16		36.5	36.4	36.3
17		31.8	30.4	31.8
18		43.3	43.2	43.2
19		35.8	35.7	35.8
20		33.4	28.5	33.4
21	4.9 (dd, J=15, 5)	76.4	33.1	76.6
22		43.6	39.6	43.6
23	0.87 (d, J=6.5)	6.9	7.1	11.5
24	0.71 (s)	14.6	15.0	16.2
25	0.86 (s)	18.0	18.3	18.3
26	0.94 (s)	18.7	20.6	18.6
27	1.07 (s)	18.9	19.0	18.9
28	1.27 (s)	32.6	32.4	32.5
29	1.09 (s)	30.3	35.4	30.7
30	0.94 (s)	26.1	32.1	26.1
OCOCH <sub>3</sub>		171.3		171.4
OCOCH <sub>3</sub>	2.02(s)	21.2		21.1

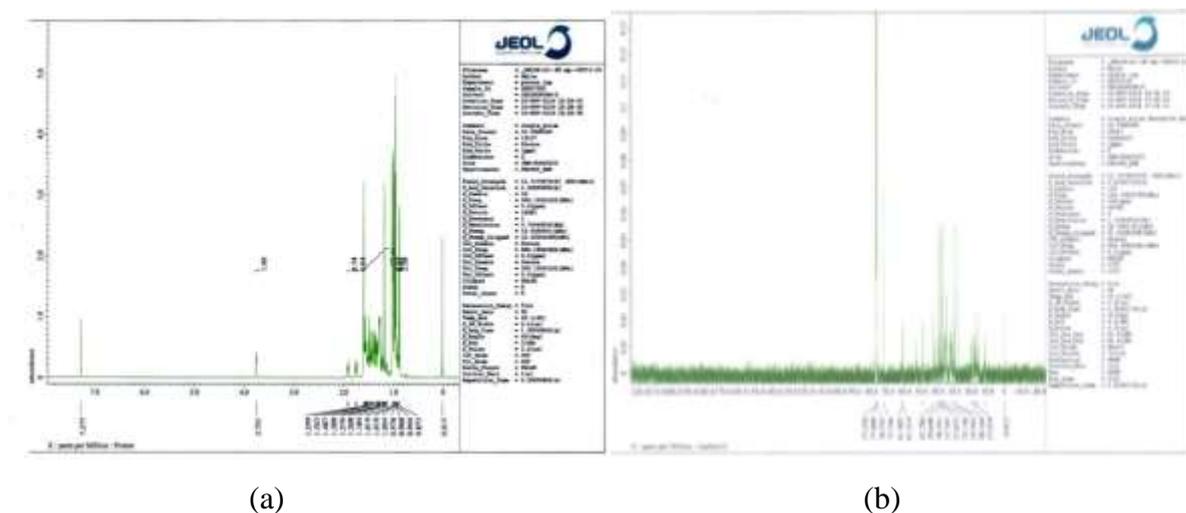
\*Patra and Chaundhuri 1987 and \*\*Anjaneyulu, *et al.*, 1993



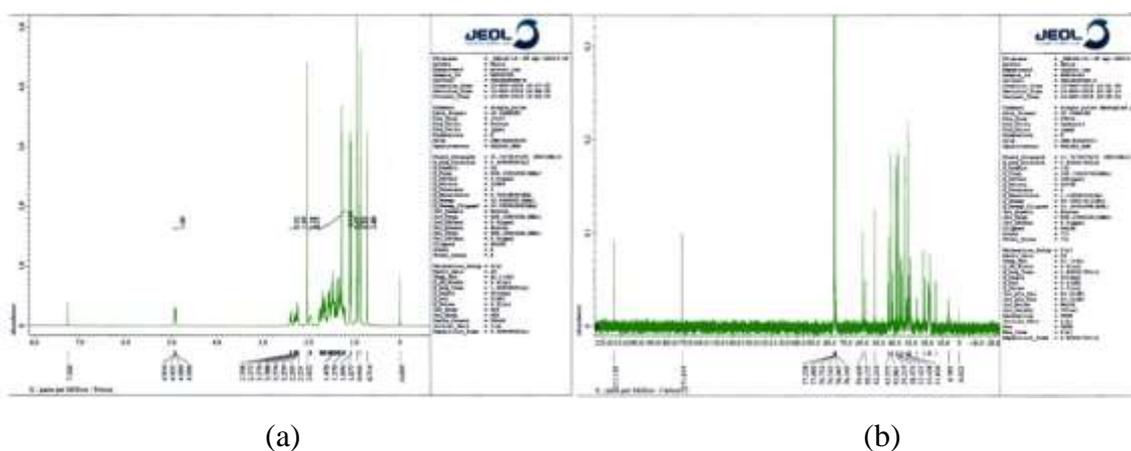
**Figure 4.** FT IR spectra of isolated compounds; (a) L-1, (b) L-2, and (c) L-3



**Figure 5.** (a) <sup>1</sup>H NMR spectra of isolated compound L-1 (500 MHz, CDCl<sub>3</sub>) and (b) <sup>13</sup>C NMR spectra of isolated compound L-1 (125 MHz, CDCl<sub>3</sub>)



**Figure 6.** (a)  $^1\text{H}$  NMR spectra of isolated compound L-2 (500 MHz,  $\text{CDCl}_3$ ) and (b)  $^{13}\text{C}$  NMR spectra of isolated compound L-2 (125 MHz,  $\text{CDCl}_3$ )



**Figure 7.** (a)  $^1\text{H}$  NMR spectrum of isolated compound L-3 (500 MHz,  $\text{CDCl}_3$ ) and (b)  $^{13}\text{C}$  NMR spectrum of isolated compound L-3 (125 MHz,  $\text{CDCl}_3$ )

### Conclusion

Three friedelane triterpenoids were isolated from the bark of *Salix alba* L., and their structures were elucidated as 3-oxofriedelane, 3- $\beta$ -hydroxyl friedelane and 3-oxofriedelan-21-acetate by  $^1\text{H}$  and  $^{13}\text{C}$  NMR and FT IR, in combination with the reference data. According to numerous scientific studies, friedelane compounds have shown antioxidant, anticancer and anti-inflammatory properties. Thus, *in vitro* and *in vivo* studies should be conducted in the future to explore these properties.

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## Reference

- Anjaneyulu, V., J. S. Babu, H. Babu, K. Raw and J. D. Connolly. (1993). "Two D: A-Friedo- OleananeDerivatives from *Euphorbia tortills*". *Phytochemistry*, vol 33 (3), pp.647- 649
- Odeh, I.C., T.A. Tor-Anyiin, J.O. Igoli and J.V. Anyam. (2016). "In Vitro Antimicrobial Properties of Friedelin -3-one from *Pterocarpus Santalinoides* L.Herit, ex D C". *African Journal of Biotechnology*, vol.15 (14), pp.531-538
- Patra, A. and S. K. Chaudhuri. (1987). "Assignment of Carbon -13 Nuclear Magnetic Resonance Spectra of some Friedelanes". *Magnetic Resonance in Chemistry*, vol. 25 (2), pp. 95-100
- Quintansa, J.S.S., E.V. Costab, J.F. Tavaresc, T.T. Souzaa, S.S. Araujoa, C.S. Estevama, A. Barisonc, A.G.S. Cabrald, M.S. Silvad, M.R. Serafinia, and L.J. Quintans-Juniora. (2014). "Phytochemical Study and Antinociceptive Effect of the Hexanic Extract of Leaves from *Combretum Duarceanum* and Friedelin, a Triterpene Isolated from the Hexanic Extract, in Orofacial Nociceptive Protocols". *Brazilian Journal of Pharmacognosy*, vol 24 (1), pp.60-66
- Randerath, K. (1966). "*Thin Layer Chromatography*". Weinheim: 2<sup>nd</sup> Ed., Verlag Chemie, GmbH, Weinheim/Bergstr., pp. 1-291
- Radi, M.H. R. A. El-Shiekh, A. M. El-Halawany, and E. A. Sattar. (2023). "Friedelin and 3 $\beta$ -Friedelinol: Pharmacological Activities". *Brazilian Journal of Pharmacognosy*, Springer Nature, pp.1-15
- Salazar, G.C.M., G.D.F. Silva, L.P. Duarte, S.A. Vieira Filho and I.S. Lula. (2000). "Two Epimeric Friedelane Triterpenes Isolated from *Maythenus Truncata* Reiss: <sup>1</sup>H and <sup>13</sup>C Chemical Shift Assignment". *Magnetic Resonance in Chemistry*, vol.38 (11), pp. 977-980
- Silverstein, R.M. and F.X. Webster. (2005). "*Spectrometric Identification of Organic Compounds*". New York:6<sup>th</sup> Ed., John Willey and Sons Inc., pp.99-164
- Tawfeek, N., M. F. Mahmoud, D. I. Hamdan, M. Sobeh, N.I. Farrag, M. Wink, and A. M. El- Shazly. (2021). "Phytochemistry, Pharmacology Medicinal Uses Plants of the Genus *Salix*: An Updated Review". *Frontiers in Pharmacology*, vol. 12, pp.1-30