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Isolation and Elucidation Structure of 28-Hydroxy-3-Friedelanone of Nyamplung (*Callophyllum inophyllum*, Linn.) Leaves

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ABSTRACT

The isolation triterpenoid from Nyamplung (*Callophyllum inophyllum,* Linn.) leaves has been conducted. The isolation was employed by maceration using ethanol as solvent and liquids extraction using ethyl acetate. Ethyl acetate extract was partitioned successively using nonpolar solvent with hexane, dichloromethane, and diethyl ether, respectively. The diethyl ether extract was purified by column chromatography. The isolated compound of fraction D1 was obtained as white solids crystal with yield of 0.0035%. The isolated compound was determined based on the FTIR, ^{1}HNMR, ^{13}CNMR, HSQC, and HMBC spectra. The isolated compound was identified as 28-hydroxy-3-friedelanone.

Keywords: *Callophyllum inophyllum,* Linn., extraction, leaf, maceration, triterpenoid

INTRODUCTION

Indonesia is a tropical country that has a natural wealth with a variety of plants but this potential has not been fully utilized as industrial ingredients, especially medicinal plants. The Indonesian people have traditionally used various types of herbs for traditional medicinal materials either as a preventive measure or treatment against various types of diseases. The use of traditional medicine is generally only based on experience/ inheritance without knowing the chemical content in detail. Among the many medicinal plants, there is a genus Calophyllum that grows in tropical regions. One of Calophyllum species is *Calophyllum inophyllum*, Linn. In Indonesia this plant is known as Nyamplung plant and there are about 180-200 different species that are famous for bioactive compounds [1]. In the Chinese state, this plant is also used as a traditional treatment to treat eye diseases, gunshot wounds, rheumatism, and inflammation and wound healing potential of methanolic extract of *Calophyllum inophyllum* Linn. Bark [2].

The isolated *C. Inophyllum*, Linn. compounds were coumarin [3], xanthones [4], flavonoids [5], triterpenoids [1], [3], [6], steroids [7]. Based on research that has been done on the leaves, roots, and wood of *C. Inophyllum*, Linn. plants have biological activities such as anti-bacteria [8], anti-oxidants [9], anti-HIV [10], anti-cancer [11], anti-inflammatory [12]. According to [13], triterpenoid compounds isolated from the leaves and stems *C. Inophyllum*, Linn provides Hela-60 cancer cell inhibitory and leukemia. A recent study of triterpenoid derived compounds isolated from *C. Inophyllum*, Linn. leave is a group of friedelin and canophilic acid [3], [6].

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The latest triterpenoid compound isolation was performed by Praveena et al. [3] and Ragasa et al. [6] using different samples and solvents. At the beginning to obtain the extract, Praveena [3] used a maceration method for 7 days using a methanol solvent, meanwhile Ragasa et al. [6] using a dichloromethane solvent with shaking for 3 days. The leaf samples of Praveena et al. [3] used *C. Inophyllum* were collected from India, meanwhile Ragasa et al. [6] using sample collected from the Philippines. The existence of different samples and solvents most probably also affect the isolated compounds. This study was conducted to isolate and elucidate triterpenoid compounds from plant leaves of *C. inophyllum* Linn originating from Surakarta, Central Java. Thus, it can add insight into the isolation of compounds from the nonpolar fraction of *C. inophyllum* Linn leaf and provide information on the content of triterpenoid compounds.

EXPERIMENT

Chemicals

Material used in this research were leaves of *C. inophyllum*, Linn., collected from Surakarta, Central Java. The solvent used for maceration is ethanol. The solvents used for liquid-liquid extraction are ethyl acetate, distilled water, hexane, dichloromethane, and diethyl ether. All solvents used were from e-Merck. The stationary phase on column gravity chromatography is Merck Kieselgel 60 (0.2-0.5 mm) silica gel.

The tools use study in this research were thin layer chromatography (TLC) analysis using silica-coated aluminum plate (Merck Kieselgel 60 GF254 0.25 mm), UV lamp with λ 254 and λ 365 nm.

FTIR analysis

Functional group identification was conducted using infrared spectrophotometer (Shimadzu type FT-IR-8201 PC) using potassium bromide pellet.

NMR analysis

The analysis of proton and carbon of the sample was performed using nuclear magnetic resonance spectrometer (Agilent 400 MHz spectrophotometer, $CDCl_3$ solvent). Normal 1D analysis for ^{1}H and ^{13}C, and 2D correlation spectroscopy method was applied for HSQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple bond correlation) spectroscopy.

Isolation procedure

A total of 400 grams of dry powdered leaves of *C. inophyllum.* Linn were macerated using 2.4 L ethanol at room temperature for 3x24 hours. Furthermore, the solvent was evaporated to obtain the ethanol extract. The ethanol extract was diluted with distilled-water and then extracted furthermore with ethyl acetate. The ethyl acetate fraction was then evaporated until a concentrated ethyl acetate extract was formed. Concentrated ethyl acetate was dissolved in distilled water and then extracted with n-hexane. The remining a distilled-water 1 fraction was taken, then extracted with dichloromethane. The distilled-water 2 fraction was taken then extracted with diethyl ether. A total of 1.3 g of diethyl ether fraction were fractionated using gravity column chromatography using Kieselgel 60 (0.2-0.5 mm) stationary phase of 36 g. The eluent used was gradient eluent system of hexane, hexane : ethyl acetate (9.5 : 0.5), hexane : ethyl acetate (9 : 1), hexane : ethyl acetate (8.5 : 1.5), hexane : ethyl acetate (8 : 2), hexane : ethyl acetate (7 : 3), hexane : ethyl acetate (6 : 4), hexane : ethyl acetate (5: 5),

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each in 100 mL. The previous sample was impregnated with silica gel of 2.6 g. Then, it was poured in column and undergone a solvent elution. The results of fractionation were checked by TLC using n-hexane : ethyl acetate (7.5 : 2.5) as eluent, and monitored under UV lamp at λ 254 and 365 nm. This step was able to obtain 8 major fractions, which are fraction A (24 mg), B (12 mg), C (9 mg), D (94 mg), E (669 mg), F (41 mg), G (226 mg), and H (200 mg).

Further purification of fraction D using gravity column chromatography (diameter 1 cm) with Kieselgel 60 (0.2 - 0.5 mm) as stationary phase 10 g, and solvent of n-hexane : ethyl acetate (8 : 2) in 100 mL, produce 2 fractions (fraction D1 and fraction D2). The D1 fraction was isolated as a white crystalline solid with Rf 0.57 and m.p > 247. In the FT-IR spectra of compound D1 shows several peaks, i.e. 3381-3546; 2866; 2881; 2930; 1707; 1465; 1388; 1122; and 1054 cm^{-1}.

RESULT AND DISCUSSION

Analysis of FT-IR.

The FT-IR spectra of the D1 compound exhibit a typical absorption-uptake in some wavelength regions (Figure 1). The spectra show the presence of hydroxyl group band at 3381-3546 cm^{-1} with a sharp uptake indicating an intermolecular hydrogen bond or a free hydrogen bond. The presence of hydroxyl groups is reinforced by the presence of C-O band in 1054 and 1122 cm^{-1}.

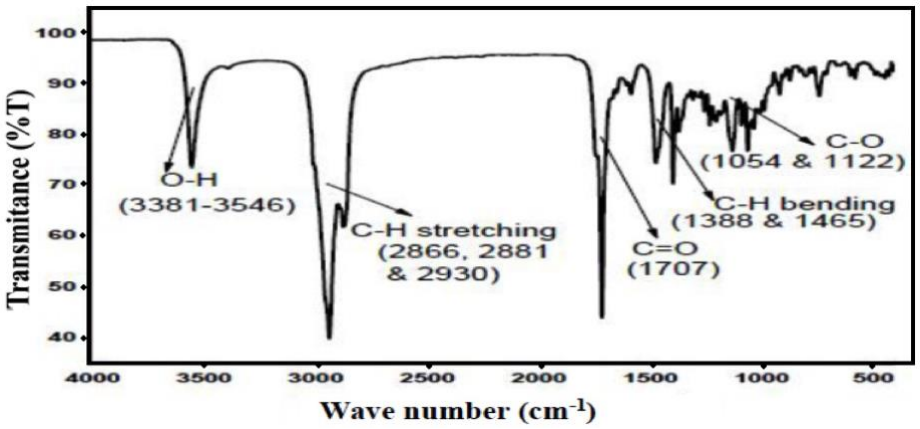

Figure 1. FT-IR spectra of the D1 compound

The D1 compound also indicate the presence of a carbonyl group (C=O) appearing at the peak at 1707 cm^{-1}. This value generally a band absorption of carbonyl from ketone. In addition, the spectra also record several peaks in 2866, 2881, and 2930 cm^{-1}. These are characteristic vibration band for alkyl groups due to C-H symmetric and asymmetric stretching. Meanwhiles, the bending peaks are recorded in 1388 and 1465 cm^{-1}. The FT-IR data shows the presence of C-H alkanes, ketone groups, and alcohols.

NMR analysis

The isolate compound fraction D1 was analyzed in a $CDCl_3$ solvent using NMR spectroscopy which included ^1H NMR, ^{13}C NMR, HSQC and HMBC. The ^1H NMR spectra (Figure 2) showed 23 proton signals that can be analyzed by multiplicity and the coupling constant (*J*), depicted in Table 1. The seven protons come from the methyl proton at δ_H (ppm) 0.71 (3H, s), 0.85 (3H, s), 0.87 (3H, d), 0.90 (3H, s), 0.97 (3H, s), 0.98 (3H, s), and 1.12 (3H,

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s) ppm. Four peaks of methyl (CH) are at 1.38 (1H, m), 1.40 (1H, m), 1.51; 1.55 (1H, m), 2.24 (1H, m). Eleven methylene protons are present in a shift between δ_H 1.27-1.96 ppm (m, 2H). One proton of CH_2 binding the OH group is 3.62 (2H, s) ppm. Meanwhile, the ^{13}C-NMR spectra are showed in Figure 3. There 30 carbons can be identified from the spectra. The detail chemical shift of each are summarized in Table 1. The spectra indicate the presence carbon for carbonyl group in 213.48 ppm, and no carbon-carbon double bond of olefin nor aromatic was recorded. Carbon bond heteroatom such as C-O was detected in between 50-70 ppm. The remaining was chemical shift for alkyl substituents such as -CH_3, CH_2, CH, and tert-alkyl.

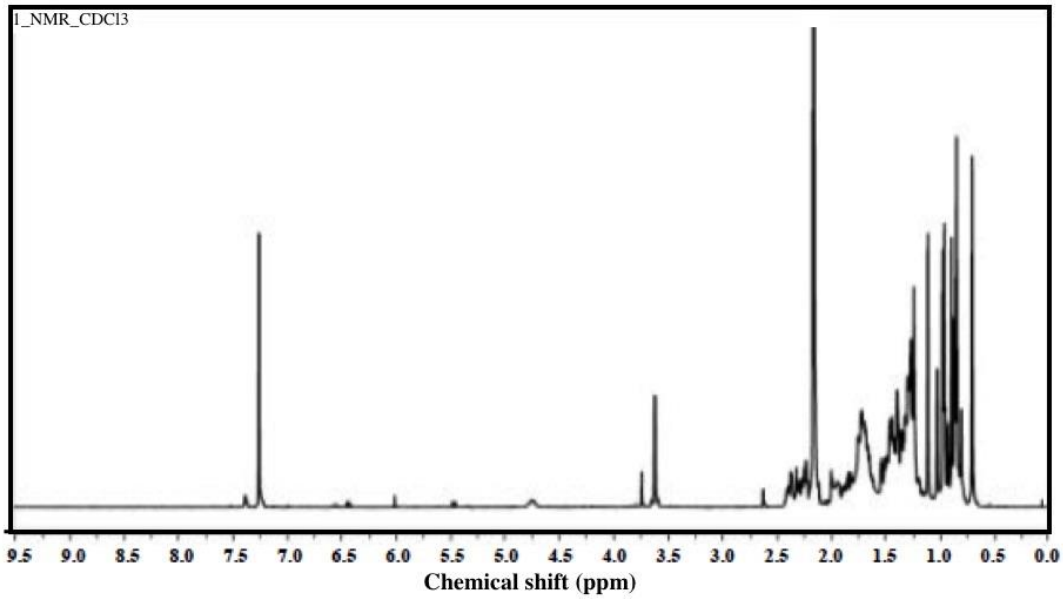

Figure 2. The ^{1}H NMR spectra of D1 molecule

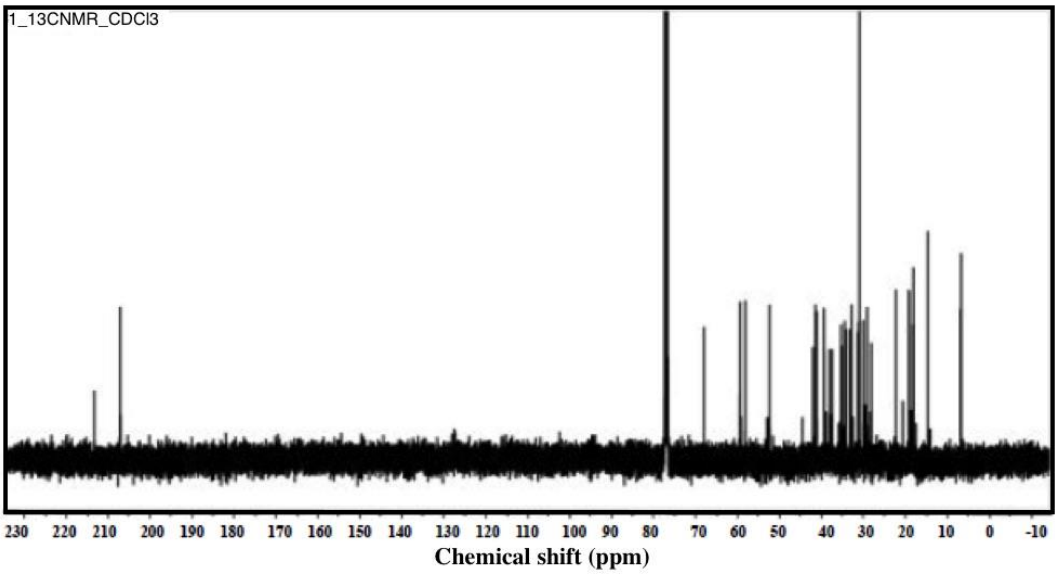

Figure 3. The ^{13}C NMR spectra of D1 molecule

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Tabel 1. Tabulation of 1D (^{1}H and ^{13}C) and 2D NMR (HSQC and HMBC) for compound D1

No.	δ_H (ppm) ($\sum$H, *m*)	δ_C (ppm), HSQC	HMBC
1	1.66;1.96 (2H, *m*)	22.40	C2, C5
2	2.37; 2.27 (2H, *m*)	41.63	C10, C3, C1
3	-	213.48	-
4	2.24 (1H, *m*)	58.32	C23, C24, C5
5	-	42.24	-
6	1.72;1.75 (2H, *m*)	41.33	C8, C10
7	1.46 (2H, *m*)	18.35	C5, C25
8	1.40 (1H, *m*)	52.57	C6, C7, C14
9	-	37.56	-
10	1.51;1.55 (1H, *m*)	59.55	C1, C5, C24, C27
11	1.44; 1.47 (2H, *m*)	35.53	C14, C25
12	1.32; 1.36 (2H, *m*)	30.20	C11, C14, C27
13	-	39.47	-
14	-	38.26	-
15	1.45; 1.31 (2H, *m*)	31.48	C13, C16, C18
16	1.82; 1.85 (2H, *m*)	29.24	C15, C17, C28
17	-	35.26	-
18	1.28 (1H, *m*)	39.55	C19, C20, C22
19	1.30 (2H, *m*)	33.46	C16
20	-	28.28	-
21	1.39 (2H, *m*)	31.31	C11, C19, C20, C28
22	1.27; 1.45 (2H, *m*)	34.59	C19, C20, C28
23	0.87 (3H, *d*)	6.97	C3, C5
24	0.71(3H, *s*)	14.79	C4, C5, C6, C10
25	0.85 (3H, *s*)	18.21	C4, C8, C10, C11
26	0.90 (3H, *s*)	19.19	C8, C9, C10, C11
27	1.12 (3H, *s*)	19.34	C12, C13, C14
28	3.62 (2H, *s*)	68.14	C16, C17
29	0.98 (3H, *s*)	34.37	C20, C22
30	0.97(3H, *s*)	32.98	C19, C20

The correlations between the carbon and the protons shown in the HSQC spectra shown in Figure 4. Based on Figure 4 can be identified that H23 predicted as methyl at δ_H 0.87 ppm correlated to carbon at δ_H 6.97 (C3) and δ_H 0.71 ppm (C5), and also correlated to carbon at δ_C 14.79 ppm (C4). Besides that, proton H25 detected at δ_H 0.85 ppm correlated with carbon at δ_C 18.21 ppm (C8). Proton at δ_H 0.90 ppm correlated to carbon at δ 19.19 ppm (C9), and proton H27 δ_H 1.12 ppm correlated with carbon at δ_C 19.34 ppm, proton at δ_H 0.98 ppm correlated with carbon at δ_C 34.37 ppm, δ_H 0.97 ppm correlated with carbon at δ_C 32.98 ppm. The methylene proton directly bonded to OH with δ_H 3.62 ppm correlates with carbon at δ_C 68.14 ppm.

The hydrogen-carbon correlation also analyzed using HBMC method, and the spectra is depicted in Figure 5. Summary of the data in Table 1. This method allow prediction until 2-3 proton-carbon or a long-range correlation. Proton at δ_H 1.66-1.96 ppm has correlation to C2

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(δ_C 41.63 ppm) and C5 (δ_C 42.24 ppm). Moroever, the proton H2 δ_H 2.37-2.27 ppm shows correlates signal to carbon of carbonyl δ_C 231.48 ppm, C10 (δ_C 59.55 ppm) and C1 (δ_H 22.40 ppm). This in agreement to the molecule of 28-hydroxy-friedelan-one [14]. The other important finding was correlation 2 proton H28 with δ_H 3.62 ppm and correlates to signal carbon C16 (δ_C 29.24 ppm) and C17 (δ_C 35.25 ppm). H28 is attached directly to hydroxyl group and the position close to and interact with C16 and C17. But position of this carbon away from C22 and C18 (Figure 6).

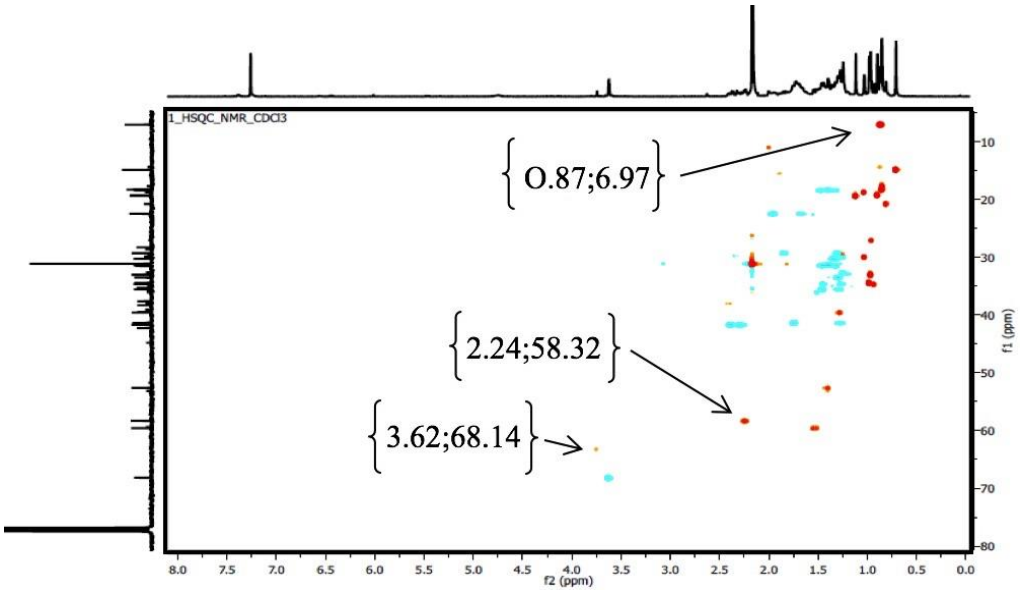

Figure 4. The HSQC spectra of molecule D1

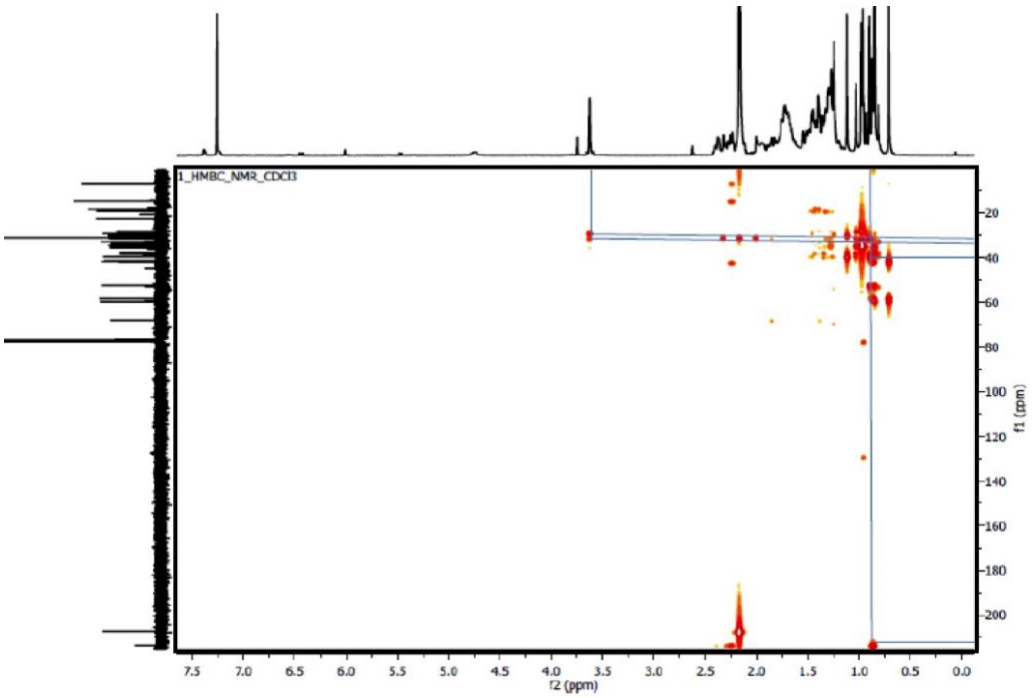

Figure 5. The HMBC spectra of molecule D1

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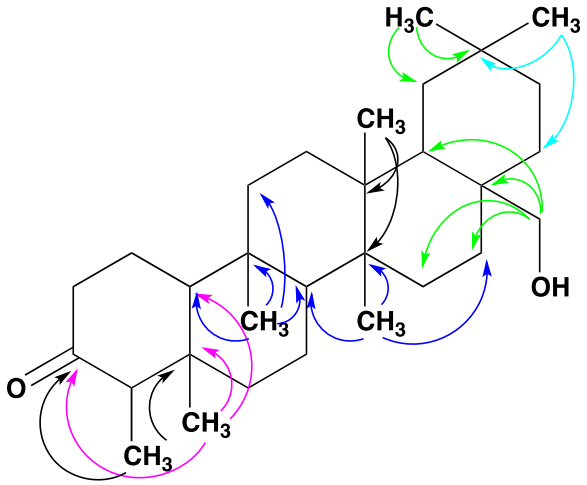

Figure 6. The correlation between proton and carbon from HMBC data

The detailed of the chemical shift comparison of ^{13}C-NMR spectra from the reference compound 28-hydroxy-3-friedelanone [14] to the chemical shift of isolated molecule D1 is visualized in Figure 7. Three important δ_C of carbon atom relate to carbonyl and its carbon-α, and carbon attached to the hydroxyl group. Both are clearly detected in the FT-IR spectra (Figure 1). The δ_C of carbonyl detected at 213.48 ppm, while the reference recorded at δ_C 212.73 ppm. Secondly, δ_C of carbon at α-position from carbonyl group, was recorded at δ_C 41.63 ppm, while the reference δ_C reported at 41.48 ppm. And lastly, carbon atom adjacent to the hydroxyl group. It was detected in δ_C 68.08 ppm and the reference reported in δ_C 68.14 ppm. Those δ_C resemble, and it can be summarized that the compound D1 isolated from the leaves of *C. inophyllum*. Linn is 28-hydroxy-friedelan-3-one (Figure 8).

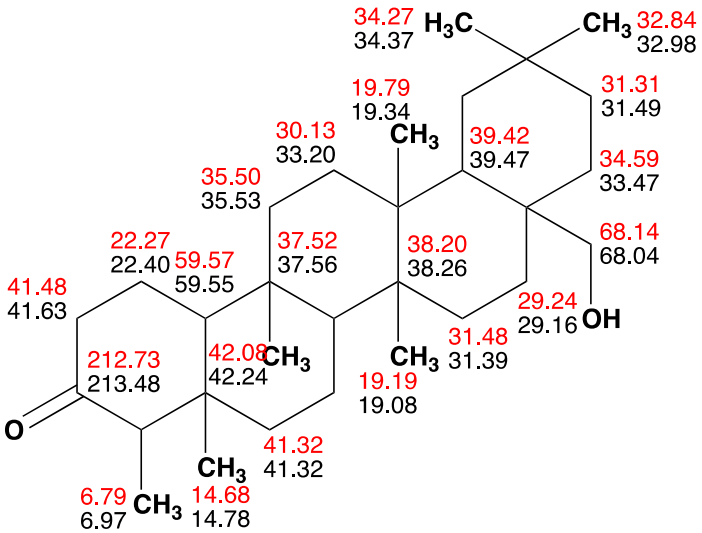

Figure 7. Comparison of carbon chemical shift (δ_C) ^{13}C-NMR of the isolated compound D1 (in black) and 28-hydroxy-friedelan-3-one (in red) [14].

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Other reference previously reported 28-hydroxy-friedalan-3-one from *Euonymus hederaceus*, belong to the family of Celastraceae [15]. Included its oxidized form in C29, namely 28-hydroxyl-friedelan-3-one-29-oic acid (Figure 9). The stereochemistry of the carbon atom was assigned based spectroscopy data (HMQC and HMBC) and the reference cited [16-17].

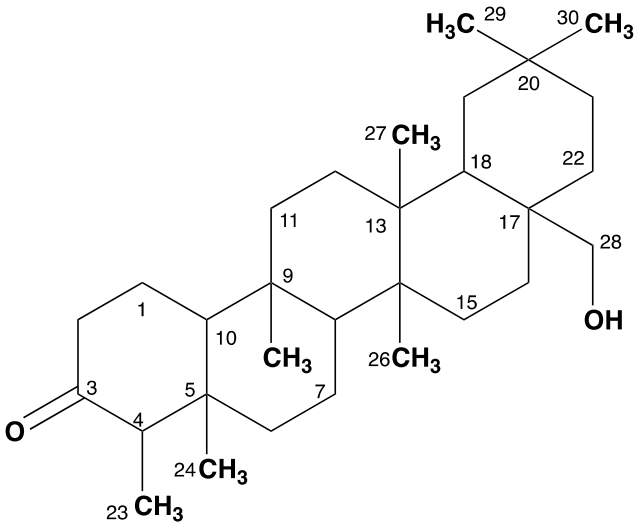

Figure 8. The molecular structure of D1 was assigned as 28-hydroxy-friedalan-3-one [14]

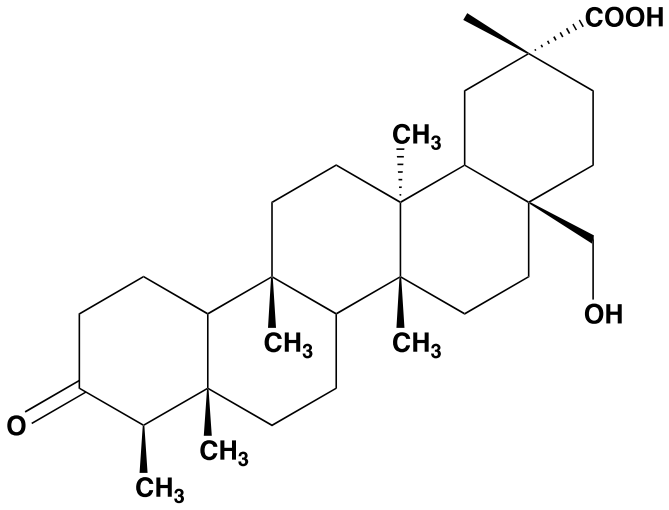

Figure 9. The molecular structure of 28-hydroxy-3-friedelan-3-one-29-oic acid [15]

CONCLUSION

The 28-hydroxy-3-friedelanone was isolated from *C. Inophyllum*, Linn. leaves as white solids crystals with yield of 0.0035%. The structure was confirmed by FTIR, ^{1}HNMR and ^{13}CNMR.

CONFLICT OF INTERESTS

Authors declare that there is no competing interest.

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