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

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ABSTRACT

The isolation triterpenoid from Nyamplung (Calophyllum 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, ¹HNMR, ¹³CNMR, HSQC, and HMBC spectra. The isolated compound was identified as 28-hydroxy-3-friedelanone.

Keywords: Calophyllum 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-Merk. 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 $\lambda$ 254 and $\lambda$ 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 remaining 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),...
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](image)

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 \(^1\)H NMR, \(^{13}\)C NMR, HSQC and HMBC. The \(^1\)H 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 \(\delta_H\) (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,
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 δH 1.27-1.96 ppm (m, 2H). One proton of CH₂ binding the OH group is 3.62 (2H, s) ppm. Meanwhile, the ¹³C-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 -CH₃, CH₂, CH, and tert-alkyl.

Figure 2. The ¹H NMR spectra of D1 molecule

Figure 3. The ¹³C NMR spectra of D1 molecule
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 δH 0.87 ppm correlated to carbon at δC 6.97 ppm (C3) and δH 0.71 ppm (C5), and also correlated to carbon at δC 14.79 ppm (C4). Besides that, proton H25 detected at δH 0.85 ppm correlated with carbon at δC 18.21 ppm (C8). Proton at δH 0.90 ppm correlated to carbon at δC 19.19 ppm (C9), and proton H27 δH 1.12 ppm correlated with carbon at δC 19.34 ppm, proton at δH 0.98 ppm correlated with carbon at δC 34.37 ppm, δH 0.97 ppm correlated with carbon at δC 32.98 ppm. The methylene proton directly bonded to OH with δH 3.62 ppm correlates with carbon at δC 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 δH 1.66-1.96 ppm has correlation to C2
Moreover, the proton H2 $\delta_H$ 2.37-2.27 ppm shows correlates signal to carbon of carbonyl $\delta_C$ 231.48 ppm, C10 ($\delta_C$ 59.55 ppm) and C1 ($\delta_H$ 22.40 ppm). This in agreement to the molecule of 28-hydroxy-friedel-one [14]. The other important finding was correlation 2 proton H28 with $\delta_H$ 3.62 ppm and correlates to signal carbon C16 ($\delta_C$ 29.24 ppm) and C17 ($\delta_c$ 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).
The detailed 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 δC 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 δC of carbonyl detected at 213.48 ppm, while the reference recorded at δC 212.73 ppm. Secondly, δC of carbon at α-position from carbonyl group, was recorded at δC 41.63 ppm, while the reference δC reported at 41.48 ppm. And lastly, carbon atom adjacent to the hydroxyl group. It was detected in δC 68.08 ppm and the reference reported in δC 68.14 ppm. Those δC 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 6. The correlation between proton and carbon from HMBC data

Figure 7. Comparison of carbon chemical shift (δC) $^{13}$C-NMR of the isolated compound D1 (in black) and 28-hydroxy-friedelan-3-one (in red) [14].
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 (HMOC and HMBC) and the reference cited [16-17].

![Figure 8](image8.png) **Figure 8.** The molecular structure of D1 was assigned as 28-hydroxy-friedalan-3-one [14]

![Figure 9](image9.png) **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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