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Research Article

Isolation of Lauric Acid and Caffeine From Durio kutejensis Root Bark

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Abstract.
Indonesia especially in Kalimantan has many herbs that are used by the people as traditional medicine such as pampaken (Durio kutejensis) and secondary metabolite in the root bark has potential to be explore. However, the study of Durio kutejensis root bark has not been reported. The objectives of this study were to isolate and identify secondary metabolite compounds of Durio kutejensis root bark. Secondary metabolite compounds of Durio kutejensis root bark were obtained with extraction, fractionation and purification process while identification with LCMS, 1D NMR, and 2D NMR analysis. The results were showed that the Durio kutejensis root bark contained lauric acid and caffeine.

Keywords: Lauric acid; caffeine; herbs; traditional medicine

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1. Introduction

Natural products are part of alternative medicine and support in the world of health with various applications [1]. Some of the drugs used are derived from natural ingredients based on empirical use

[2]. The process of extracting natural products is carried out through extraction and isolation so that solvents and isolation techniques are very important [3][4]. The isolation of secondary metabolites in a natural resource like a plant is carried out through a long and quite complicated process with a variety of methods that have advantages and disadvantages. There are hundreds or thousands of compounds contained in plant samples so it is necessary to pay attention to the amount of availability, pharmacological functions, and characteristics of each compound. One of the plants on the island of Kalimantan which is included in the genus Durio, namely Durio kutejensis with limited research and references has potential for herbal medicine. Durio kutejensis is known as Lai, durian kenyak, durian pulu, paken, and pampaken in Kalimantan. People usually

use fruits and its flower for consumption and traditional medicine [5]. Durio kutejensis contains terpenoid, tannin, and phenols [6][7][8]. Research shows that stem bark of Durio kutejensis has many metabolites and promisingly for pharmacological test [9]. Based on previous research, Durio kutejensis leaf was tested its activity as an antioxidant [10][11] and stem bark of Durio kutejensis has potential as antidiabetic activities [12]. There are many benefits to Durio kutejensis as a natural resource because it is important to know its secondary metabolite and also its pharmacological activities. Interestingly, there is no research before on root bark for secondary metabolite and its activity. Then, the study recently aimed to investigate and isolate the secondary metabolites of Durio kutejensis root bark such as lauric acid and caffeine. Lauric acid is known to be contained in various plants such as coconut and has antibacterial effects [13]. Caffeine compounds in many plants such as tea, coffee, and other plants [14]. Therefore, it is important to carry out various secondary metabolic compounds including lauric acid and caffeine.

2. Methods

2.1. Material

Durio kuetenjesis root bark, ethanol 96%, filter paper whattman, chloroform, kloroform-d (CDCl3), aquadest, ethyl acetate, methanol, hexane, Silica Gel TLC plate (merck kieselgel 60 GF254 0,25 mm), silica gel (Silica gel with gypsum Merck 7749), silica gel (merck Sie-gel 60 GF254), silica gel impregnation (merck kieselgel 60 GF254 0,2-0,5 mm), Silica gel 60 RP-18 F_{254} (merck).

2.2. Procedures

2.2.1. Extraction and Fractionation

The root bark of Durio kutejensis was collected from Pulang Pisau, Central Kalimantan and determined at Laboratory of Biology Department, Universitas Negeri Sebelas Maret (No.209/UN27.9.6.4/Lab/2017). The root bark was cleaned, chopped, dried in direct sunlight, and powdered. Root bark (5 Kg) is extracted with ethanol 96% for 3 days by the maceration method. The filtrate was filtered and evaporated with a rotary evaporator at 50˚C. The chloroform fraction was obtained by immersing 50 grams of ethanol extract in total 500 mL of chloroform solvent (three times of partition).

2.2.2. Isolation and compound identification

Purification of the chloroform fraction was carried out using sephadex chromatography and

preparative TLC. The results of TLC profile showed that the fraction 2A (1 gram) was purified with Sephadex 20-LH (GE) exclusion chromatography. Column specifications are 50 cm (long), 3 cm (wide), 25 cm (height) and ethanol 96% as mobile phase. Separation of fraction is observ by color separation on the column. The results of the sub-fraction were checked for TLC profiles in UV lamps (254 nm and 365 nm). Solution fractions with similiar profile or Rf value are combined and evaporated. The sub-fraction of sephadex was further purified using preparative TLC with 0.25 mm GF254 silica gel as stationary phase and the optimized mobile phase. The results of the stain separation of the compound are scraped off and separated from the stationary phase using methanol: chloroform (7:3) solvent in the separating column.

The purity of the isolates was checked using TLC normal phase silica gel GF254 0.25 mm and TLC reverse phase Silica gel 60 RP-18 F_{254}. Isolate compounds from chloroform fraction were analyzed using 1D NMR (1H NMR and 13C NMR), 2D NMR (COSY, HMQC, and HMBC) and LC-MS. Isolate compounds from the chloroform fraction were analyzed using NMR (JEOL ECA 400) running at 400MHz for 1H NMR and 100 MHz for 13C NMR and CDCl3 solvent. 2D NMR analysis (COSY, HMQC, and HMBC) was performed after the 1H NMR and 13C NMR analyzes were complete. Then, the isolates were analyzed using LC-MS (Waters XEVO TQD). The sample was first dissolved in methanol and filtered using a 0.2 µm PTFE filter (Advantec). The mobile phase used methanol : water (9:1) and the stationary phase used column C-18 (RP Cosmosil) with a size of 150 mm x 4.6 mm and scanning at 100-700 m/z.

3. Results and Discussion

3.1. Extraction and Fractionation

The chloroform fraction was 13.1 grams. After that, the chloroform fraction was checked for the separation profile of the compound with the mobile phase of chloroform : ethyl acetate (9:1) and 10

grams of the chloroform fraction was fractionated again using Vacuum Liquid Chromatography

(VLC). The results of the weight of each VLC fraction are fractions 1A (2.06 g), 1B (5.99 g), 2A (1 g),

2B (19 mg), 3A (6 mg), 3B (3 mg), 4A (10 mg), and fraction 4B (38 mg).

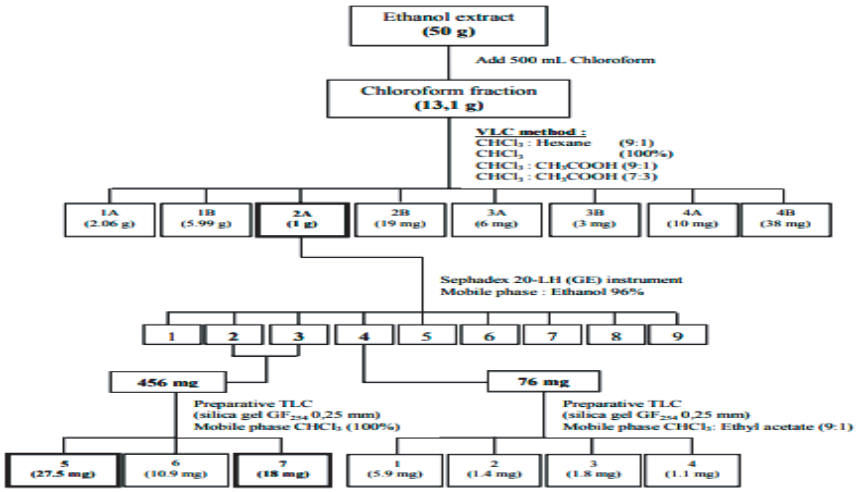

Figure 1: Isolation process of lauric acid (5) and caffeine (7) from Durio kutejensis root bark.

The isolation process in Figure 1 shows that the results of purification of the chloroform fraction from fraction 2A obtained 9 sub fractions that were checked for separation profiles at UV 254 nm and 365 nm. The combined sub fraction 2 and 3 (456 mg) and sub fraction 4 (76 mg) were selected for further purification using preparative TLC with chloroform: ethyl acetate (9:1) as mobile phase for sub fraction 4 and 100% chloroform mobile phase for sub combined fraction (2 and 3). The results of sub- fraction 4 purification obtained isolate 1 (5.9 mg), isolate 2 (1.4 mg), isolate 3 (1.8 mg) and isolate 4 (1.1 mg). While the combined purification results of sub fractions 2 and 3 obtained isolate 5 (27.5 mg), isolate 6 (10.6 mg), and isolate 7 (18 mg).

3.2. Isolation and Compound Identification

3.2.1. Lauric acid

The 1H NMR spectrum of isolate 5 was measured at a frequency of 400 MHz and the 13C NMR spectrum was measured at a frequency of 100 MHz using CDCl3 solvent. The results of 1H NMR and 13C NMR spectrum analysis for isolate 5 are shown in Table 1. The 1H NMR spectrum showed the presence of a methyl group (CH3) in isolate 5 which appeared in the chemical shift δH 0.87 ppm (t, 2H). Chemical shift δH 1.24 ppm (s, 16H); δH 1.62 ppm (q, 2H) and δH 2.33 ppm (t, 2H) indicate the presence of a methylene (CH2) group. The 1H NMR spectrum did not show any peaks in the

presence of carboxylates (COOH) which was probably due to the effect of using CDCl3 solvent. The 13C NMR spectrum showed that isolate 5 contained 12 carbon atoms which appeared at δC 14.22; 22.79; 24,80; 29.16; 29.34; 29.45; 29.53; 29.68; 29.78; 32.02; 33.94 as an aliphatic chain and 178.69 ppm as carboxylate. Therefore, it is possible that isolate 5 belongs to the group of saturated fatty acid compounds, namely lauric acid (C12H24O2). 2D NMR analysis was carried out to determine the relationship or correlation between protons-protons and the bonds between protons-carbon as shown in Table 1 and Figure 2.

HMQC analysis showed a correlation between carbon and proton atoms seen in the chemical shift of δC 14.22 ppm with δH 0.87 ppm in the form of a methyl group. The methylene group was shown by the correlation of chemical shear δC 29.68 and 29.78 ppm with δH 1.24 ppm; δC 24.80 ppm with δH 1.62 ppm; δC 33.94 ppm with δH 2.33 ppm. COSY analysis showed a correlation between proton and proton atoms between the chemical shift δH 0.87 ppm and δH 1.24 ppm; δH 1.62 and δH 02.33 ppm adjacent to each other. HMBC analysis shows a 2-3 bond correlation between proton and carbon atoms seen in the chemical shift of δH 0.87 ppm with δC 22.79 and 32.02 ppm. Chemical shift δH 1.24 ppm correlates with δC 29.34; 29.45 and 29.53 ppm. Chemical shift δH 1.62 ppm correlates with δC 29.16 and 178.69 ppm. The chemical shift δH 2.33 ppm correlates with δC 24.80; 29, 16 and 178.69 ppm. The LC-MS (ES +) spectrum analysis of isolate 5 showed the molecular weight of isolate 7 was 200.62. Based on the analysis of 1D NMR, 2D NMR and LC-MS spectrum data, it can be concluded that isolate 5 is a lauric acid. The results of 1H NMR and 13C NMR spectrum analysis for isolate 5 were confirmed in [15] and [16] showed that isolate 5 is a lauric acid with 12 carbon pieces in the form of methyl and methylene which compose the aliphatic chain and 1 carboxylate group.

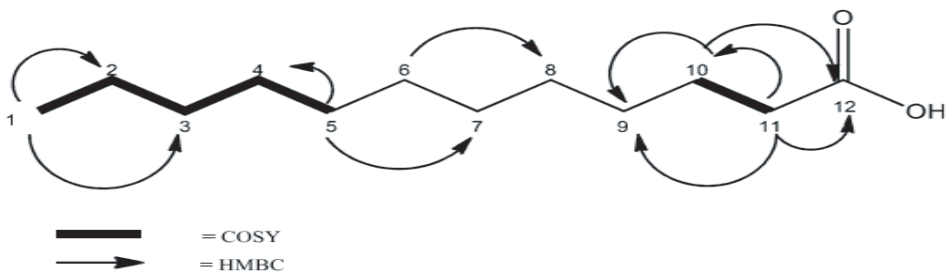

Figure 2: Correlation analysis of isolate 5.

Table 1: 1D NMR and 2D NMR analysis of isolate 5.

No.	δC	δH (Multiplicity, *J*)	HMBC
1.	14.22	0.87 (*t*, J = 6.8 Hz)	H-1, C-2, C-3
2.	22.79	-	-
3.	32.02	-	-
4.	29.45	-	-
5.	29.78	1.24 (*s*)	H-5, C-4, C-7
6.	29.68	1.24 (*s*)	H-6, C-8
7.	29.53	-	-
8.	29.34	-	-
9.	29.16	-	-
10.	24.80	1.62 (*q*, J = 7.2 Hz)	H-10, C-9, C-12
11.	33.94	2.33 (*t*, 7.2 Hz)	H-11, C-9, C-10, C-12
12.	178.69	-	-

3.2.2. Caffeine

The 1H NMR spectrum of isolate 7 was measured at 400 MHz and the 13C NMR spectrum was measured at 100 MHz using CDCl3 solvent. The results of 1D NMR (1H NMR and 13C NMR) and 2D NMR (HMQC, COSY, HMBC) spectrum analysis are shown in Table 2. The 1H NMR spectrum of isolate 7 showed the presence of a methyl group (CH3) at δH 3.40; 3.57; 3.98 ppm in the form of a singlet signal and the metine (CH) group in the heterocyclic on the chemical shift δH 7.51 ppm in the form of a singlet signal so that it is known that there are 10 H atoms in isolate 7. The 13C NMR spectrum shows that isolate 7 has three CH3 that appear in the chemical shift δC 28.06; 29.90 and 33.74 ppm. In addition, there is a double bond carbon in the chemical shift δC 107.71; 141.49; 148.70; 151.82 and 155.53 ppm so it is known that there are a total of 8 carbon atoms in isolate 7. Therefore, it is possible that isolate 7 is a purine alkaloid compound, namely caffeine ($C_8H_{10}N_4O_2$). 2D NMR analysis namely HMQC, COSY and HMBC was carried out on isolate 7 to determine the relationship or correlation between protons and protons and the bonds between protons and carbon as shown in Table 2 and Figure 3.

HMQC analysis shows that there is a correlation between carbon atoms and nitrogen atoms which is seen in the chemical shift of δC 28.06 ppm correlates with δH 3.40 ppm. Chemical shift δC 29.90 ppm correlates with δH 3.57 ppm and chemical shift δC 33.74 ppm correlates with δH 3.98 ppm. In addition, there is a correlation between carbon and hydrogen atoms in the chemical shift of δC 141.49 ppm to δH 7.51 ppm. The COSY analysis shows no correlation between proton atoms and proton atoms that are close to each other. HMBC analysis shows a 2-3 bond correlation between proton

Table 2: 1D NMR and 2D NMR analysis of isolate 7.

No.	δC	δH (Multiplicity, *J*)	HMBC
1.	28.06	3.40 (s)	H-1, C-2, C-6
2.	148.70	-	-
3.	29.90	3.57 (s)	H-3, C-2, C-4
4.	151.83	-	-
5.	107.71	-	-
6.	155.53	-	-
7.	33.74	3.98 (s)	H-7, C-5, C-8
8.	141.49	7.51 (s)	H-8, C-4, C-5
9.	-	-	-

atoms and carbon atoms seen in the chemical shift of δH 3.40 ppm with δC 148.70 and 155.53 ppm; δH 3.57 ppm with δC 148.70 and 151.82 ppm; δH 3.98 ppm with δC 107.71 and 141.49 ppm; δH 7.51 ppm with δC 107.71 ppm. The LC-MS (ES +) spectrum analysis of isolate 7 showed that the molecular weight of isolate 7 was 195.01. Based on the analysis of 1D NMR, 2D NMR and LC-MS spectrum data, it can be concluded that isolate 7 is a caffeine.

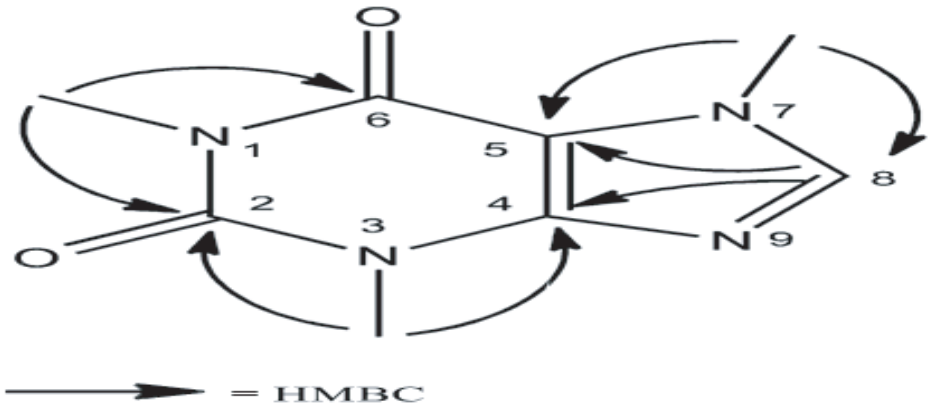

Figure 3: Correlation analysis of isolate 7.

The results of 1H NMR and 13C NMR spectrum analysis for isolate 7 were confirmed in [17] and [18] which showed a caffeine compound has carbon and nitrogen bonds in the chemical structure at positions 1, 3 and 7 and methyl bonds at position 8. Based on previous references, lauric acid and caffeine compounds have never been isolated from Durio kutejensis root bark.

4. Conclusions

In conclusion, Durio kutejensis root bark contains lauric acid and caffeine as secondary metabolite. In the next, some metabolites need to be isolated, identified and tested in several pharmacological tests.

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5. Conflict of Interests

The author declared there are no conflicts of interest.

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