

# Mutant Plant Tipobio Variety of Rodent Tuber (Typhonium flagelliforme): Fatty Acids Compounds and in Vitro Anticancer Activity

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**Abstract.** The gamma-ray irradiation has been used to increase the content in the chemical compounds of Typhonium flagelliforme with anticancer activity. This study was to determine the new bioactive compounds through <sup>1</sup>H-NMR, <sup>13</sup>C NMR spectra, and HR-TOF MS analysis. The isolated compounds from Tipobio variety of mutant plant were found bioactive compounds of fatty acid group which have the potential as anticancer activity. Two fatty acid derivatives, 2-octenoic acid (1) and 2-hexenoic acid (2) were isolated from ethyl acetate extract of the rodent tuber mutant plant (*T. flagelliforme*). The chemical structures were identified based on spectroscopic evidence and compared to previously reported spectra. Compounds (1-2) were evaluated for cytotoxic activity against MCF-7 breast cancer cells *in vitro*. The cytotoxicity activity of rodent tuber mutant plants was tested on breast cancer cell line (MCF-7) performed by MTT assay method. The cytotoxic effect of 2-octenoic acid and 2-hexenoic acid had IC<sub>50</sub> value about 2.66 µg mL<sup>-1</sup> and 3.10 µg mL<sup>-1</sup>, respectively. In this study, it was demonstrated that rodent tuber mutant plants of the Tipobio variety showed promising results as an anticancer drug.

## 1 Introduction

Typhonium flagelliforme is a rodent tuber herbal plant of the Araceae family from Bogor, Indonesia. It has demonstrated anticancer activity against various cancer cell lines, including lung and breast cancer cells [1], T4-lymphoblastoid leukemia [2], T47D breast cancer [3], and MCF-7 breast cancer [4]. The rodent tuber mutant plants have conducted research through *in vitro* biotechnology (somaclonal combination) with gamma ray irradiation to produce mutant plants. The results showed that the mutant plants have characteristics morphology changes in the number of leaves, plants height, and wide leaves [5]. The effect of gamma irradiation can also cause genetic changes or mutations. Thus, genetic changes have been detected in *T. flagelliforme* mutants of Tipobio variety in the first generation (MV1) [6], and a molecular random amplified polymorphic DNA analysis revealed genetic changes in the second-generation (MV2) and third generation (MV3) *T. flagelliforme* mutants compared with the wild type [7].

The gas chromatography-mass spectrometry (GC-MS) analysis of the chemical compounds of Tipobio variety mutant plant extract. The chemical compounds

were detected as hexadecenoic acid, octadecadienoic acid, stigmaterol, and beta-sitosterol [8]. Among these compounds, stigmaterol exhibits antioxidant activity against Ehrlich ascites carcinoma (EAC) cells by decreasing lipid peroxidation and increasing the catalase content in the liver of EAC rats [9]. It is the main phytosterol as fatty acids in various herbal plants with anti-inflammatory and anticancer properties [10]. It can inhibit the growth of uterine cancer cells ES2 and OV90 by 50% at a treatment concentration of 20 µg/mL [11]. GC-MS analysis showed that MV4 mutant leaves contained 5 higher anticancer compounds than their mother plants [12]. The superior mutant clones which have high anticancer content are KB 6-1-1-2 and KB 6-1-2, Tipobio, and KB 6-9-4 [10].

Our latest research reported that the ethanol extract of KB 6-9-5 and KB 6-1-3-4 that has been tested on MCF-7 breast cancer cells has shown to inhibit the cell growth *in vitro* [10]. The rodent tuber mutant plants extract from KB 6-9-5, and KB 6-1-3-4 showed the IC<sub>50</sub> values of 7.04 and 12.48 µg mL<sup>-1</sup> while the wild type IC<sub>50</sub> values were obtained at 19.11 µg mL<sup>-1</sup> [8]. Therefore, this study determines the cytotoxic activity, and morphological observation on MCF-7 breast cancer *in vitro* from the isolate compounds of 2-octenoic acid

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and 2-hexenoic acid from Tipobio variety of rodent tuber mutant plants.

## 2 Material and methods

### 2.1 General identification of chemical compounds

UV spectra was measured using a TECAN Infinite M200 pro, with MeOH. The IR spectra and mass spectra were recorded on a SHIMADZU IR Prestige-21 in KBr and Waters Xevo HR-TOF MS respectively. Using a JEOL ECZ-500, the NMR data was recorded at 500 MHz for  $^1\text{H}$  and 125 MHz for  $^{13}\text{C}$ , using TMS as internal standard. Column chromatography was conducted on the silica gel 60 (<70, 70–230 and 230–400 mesh, Merck), after which TLC analysis was carried out on 60 GF<sub>254</sub> (Merck, 0.25 mm) using various solvent systems, in order to detect spots by irradiating under ultraviolet-visible light (257 and 364 nm) and heating of silica gel plates, sprayed with H<sub>2</sub>SO<sub>4</sub> in N-hexane (10%).

### 2.2 Plant material

*T. flagelliforme* mutant plant Tipobio variety was collected from Sianipar & Purnamaningsih's collection. The mutant plants were obtained by irradiating with gamma rays at a dose of 6 Gy to produce mutagenesis in vitro. The mutant plant was acclimatized and maintained at the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Ministry of Agriculture, Bogor, Indonesia.

### 2.3 Extraction and isolation

The tubers of Tipobio variety were harvested and dried to obtain a powder (3.12 kg), which was then submerged in ethanol 96% and macerated seven times until the extract was colorless. The solvents were evaporated using a rotary evaporator (Rotavapor R-300, Buchi) at 50°C to obtain a highly concentrated ethanol extract (147.47 g). Using the KCV method with vacuum assistance, extraction with ethyl acetate (17.75 g) was selected for faster and more efficient separation. Separation with KCV is based on the principle of adsorption using silica gel G60 (70–230 mesh) as the stationary phase and an *n*-hexane: ethyl acetate: methanol solvent system with a 10% gradient. As a result, the ethyl acetate extract contained 21 fractions. Each fraction was analyzed by thin-layer chromatography (TLC) on a GF<sub>254</sub> silica plate eluted with *n*-hexane: ethyl acetate (7:3) resulting in eight fractions (A–H). Fraction A (1.12 g) was subjected to column chromatography on silica gel using *n*-hexane:CHCl<sub>3</sub> (5% stepwise), as eluting solvents to afford seven subfractions (A1–A7). Subfraction A3 (632.2 mg) was chromatographed on a column of silica gel, eluting with *n*-hexane: CH<sub>2</sub>Cl<sub>2</sub> (7:3), to give six subfractions (A3A–A3G). Similarly, subfraction A3D (100.1 mg) was chromatographed on silica gel eluted with *n*-hexane: CH<sub>2</sub>Cl<sub>2</sub>: EtOAc (7:2.5:0.5), to give **1** (8.1 mg). Subfraction A3E (90.2 mg) was chromatographed on

silica gel eluted with petroleum ether: CHCl<sub>3</sub> (7:2), to give **2** (5.2 mg).

#### 2.3.1 2-octenoic acid (1)

Oil yellow; HR-TOFMS *m/z* 143.1019 [M-H]<sup>+</sup> (cal. C<sub>8</sub>H<sub>15</sub>O<sub>2</sub> *m/z* 143.1094),  $^1\text{H}$  NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  0.92 (3H, t, *J* = 6.8 Hz, H<sub>3</sub>-8), 1.26–1.33 (6H, m, H<sub>2</sub>-5 – H<sub>2</sub>-7), 2.03 (2H, m, H<sub>2</sub>-4), 5.35 (1H, dd, *J* = 15.2 and 5.6 Hz, H-3), 5.77 (1H, d, *J* = 15.2 Hz, H-2);  $^{13}\text{C}$  NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  14.0 (C-8), 22.6 (C-7), 26.8 (C-6), 29.6 (C-5), 31.9 (C-4), 121.6 (C-2), 129.8 (C-3), 168.8 (C-1).

#### 2.3.2 2-hexenoic acid (2)

Oil yellow; HR-TOFMS *m/z* 115.1211 [M-H]<sup>+</sup> (cal. C<sub>6</sub>H<sub>10</sub>O<sub>2</sub> *m/z* 115.1094),  $^1\text{H}$  NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  0.92 (3H, t, *J* = 6.8 Hz, H-6), 1.33 (2H, m, H-5), 2.03 (2H, m, H-4), 5.35 (1H, dd, *J* = 15.0 and 5.6 Hz, H-3), 5.77 (1H, d, *J* = 15.0 Hz, H-2);  $^{13}\text{C}$  NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  14.4 (C-6), 22.6 (C-5), 26.8 (C-4), 122.6 (C-2), 130.1 (C-3), 167.6 (C-1).

## 3 Results and discussion

### 3.1 Chemical composition of fattyacids

The ethyl acetate extract from the leaf of *T. flagelliforme* was fractionated by column chromatography on silica gel, using a gradient of *n*-hexane, EtOAc and MeOH (10% stepwise). The fractions were repeatedly subjected to normal phase column chromatography, to accommodate compounds (**1**–**2**). **2-octenoic acid (1)** was observed as an Oil yellow, with its molecular composition established as C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>, based on HR-TOFMS. This showed a [M+H]<sup>+</sup> ion peak at *m/z* 143.1019 (calcd. C<sub>8</sub>H<sub>15</sub>O<sub>2</sub> *m/z* 143.1094), requiring two degrees of unsaturation. The  $^1\text{H}$ -NMR spectrum (Table 1) showed one primary methyl at  $\delta_{\text{H}}$  0.92 (3H, t, 6.8 Hz, H<sub>3</sub>-8), two sp<sup>2</sup> methine protons at  $\delta_{\text{H}}$  5.77 (1H, d, *J* = 15.2 Hz, H-2), 5.35 (1H, dt, *J* = 5.6; 15.2 Hz, H-3) indicates trans double bonds and four methylenes at  $\delta_{\text{H}}$  1.26–1.33 (6H, m, H<sub>2</sub>-5–H<sub>2</sub>-7), 2.03 (2H, m, H<sub>2</sub>-4). The  $^{13}\text{C}$  NMR together with the DEPT spectra revealed eight carbons consisting of a carbonyl at  $\delta_{\text{C}}$  168.8 (C-1),  $\alpha,\beta$ -unsaturated secondary at  $\delta_{\text{C}}$  121.6 (C-2) and 129.8 (C-3), four carbons methylene at  $\delta_{\text{C}}$  22.6 (C-7), 26.8 (C-6), 29.6 (C-5), 31.9 (C-4) and one methyl at  $\delta_{\text{C}}$  14.0 (C-8).

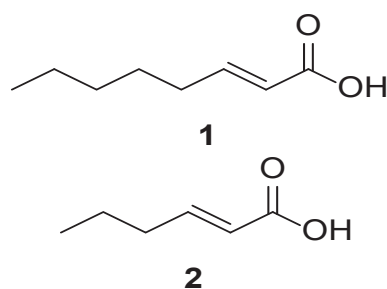
The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound **1** showed correlations in H<sub>2</sub>-H<sub>3</sub>-H<sub>4</sub>-H<sub>5</sub>-H<sub>6</sub>-H<sub>7</sub> and H<sub>8</sub>, supporting the presence of a secondary fatty acid [3]. The HMBC correlations from H-2 to C-1 and C-4; H-3 to C-1, C-2 and C-5; H-4 to C-1, C-2 and C-6, H-5 to C-3 and C-7; H-6 to C-8; H-7 to C-5 and C-8, H-8 to C-6 and C-7, which was verified by correlations observed in the  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC spectra (Figure 2). **2-hexenoic acid (2)** was observed as an oil yellow, with its molecular composition established as C<sub>6</sub>H<sub>10</sub>O<sub>2</sub>, based on HR-TOFMS. This showed a [M+H]<sup>+</sup> ion peak at *m/z* 115.1211 (calcd. C<sub>6</sub>H<sub>11</sub>O<sub>2</sub> *m/z* 115.1094), requiring two

degrees of unsaturation. The  $^1\text{H-NMR}$  spectrum (Table 1) showed one primary methyl at  $\delta_{\text{H}}$  0.92 (3H, t, 6.8 Hz, H<sub>3-6</sub>), two  $\text{sp}^2$  methine protons at  $\delta_{\text{H}}$  5.77 (1H, d,  $J=15.0$  Hz, H-2), 5.35 (1H, dt,  $J=5.6; 15.0$  Hz, H-3) indicates trans double bonds and two methylenes at  $\delta_{\text{H}}$  2.03 (2H, m, H<sub>2-4</sub>), 1.33 (2H, m, H<sub>2-5</sub>). The  $^{13}\text{C}$  NMR together with the DEPT spectra revealed six carbons consisting of a carbonyl at  $\delta_{\text{C}}$  167.6 (C-1),  $\alpha,\beta$ -unsaturated secondary at  $\delta_{\text{C}}$  122.6 (C-2) and 130.1 (C-3), two carbons methylene at  $\delta_{\text{C}}$  22.6 (C-5), 26.8 (C-4) and one methyl at  $\delta_{\text{C}}$  14.0 (C-8).

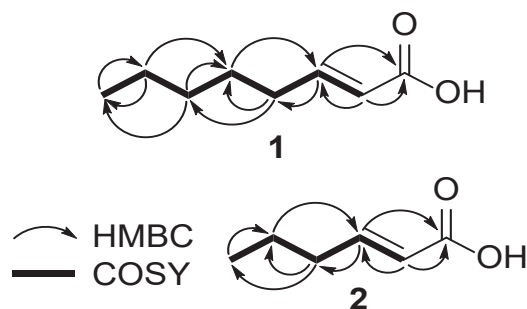
The  $^1\text{H-}^1\text{H}$  COSY spectrum of compound **2** showed correlations in H<sub>2</sub>-H<sub>3</sub>-H<sub>4</sub>-H<sub>5</sub> and H<sub>6</sub>, supporting the presence of a secondary fatty acid [3]. The HMBC correlations from H-2 to C-1 and C-4; H-3 to C-1, C-2 and C-5; H-4 to C-1, C-2 and C-6, H-5 to C-3 and C-5; H-6 to C-5 and C-4, which was verified by correlations observed in the  $^1\text{H-}^1\text{H}$  COSY and HMBC spectra (Figure 2).

**Table 1.** NMR data compound **1-2** (500 MHz for  $^1\text{H}$  dan 125 MHz for  $^{13}\text{C}$ ).

Position Carbon	1		2	
	$^{13}\text{C-NMR}$ $\delta_{\text{C}}$ (mult.)	$^1\text{H-NMR}$ $\delta_{\text{H}}$ [( $\Sigma\text{H}$ , mult, $J(\text{Hz})$ )]	$^{13}\text{C-NMR}$ $\delta_{\text{C}}$ (ppm)	$^1\text{H-NMR}$ $\delta_{\text{H}}$ [( $\Sigma\text{H}$ , mult, $J(\text{Hz})$ )]
1	168.8	-	167.6	-
2	121.6	5.77 (1H, d, 15.2)	122.6	5.77 (1H, d, 15.0)
3	129.8	5.35 (1H, dt, 15.2; 5.6)	130.1	5.35 (1H, dt, 15.0; 5.6)
4	31.9	2.03 (2H, m)	26.8	2.03 (2H, m)
5	29.6	1.26-1.33 (2H, m)	22.6	1.33 (2H, m)
6	26.8	1.26-1.33 (2H, m)	14.4	0.92 (3H, t, 6.8)
7	22.6	1.26-1.33 (2H, m)	-	-
8	14.0	0.92 (3H, t, 6.8)	-	-



**Fig. 1.** Structure of compounds (1-2).



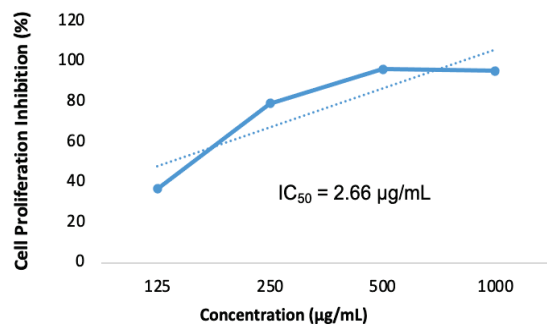
**Fig. 2.** Selected HMBC and COSY correlations for compounds (1-2).

### 3.2 Cytotoxic activity of isolated compounds (1-2) on MCF-7 cancer cell line

The results of this study revealed that 2 bioactive compounds from fatty acid have potential as anticancer activity are 2-octenoic acid and 2-hexenoic acid. The potential target as anticancer properties among 2 bioactive compounds from fatty acid is 2-octenoic acid. 2-octenoic has the most different anticancer properties. As shown Figure 3, 2-octenoic in Tipobio variety has higher IC<sub>50</sub> value about 2.66  $\mu\text{g mL}^{-1}$ . This study confirmed that Tipobio variety has potency as anticancer agent. 2-octenoic or stearic acid is a saturated fatty acid found in relatively high concentrations in some foods. 2-octenoic or stearic acid has been reported to inhibit the development of human breast cancer cells in proliferation *in vitro* [14, 15, 16] and *in vivo* [17]. Stearic acid has also been shown to induce apoptosis in breast cancer cells and inhibit cell cycle of breast tumors [18, 19]. Interestingly, epidemiological studies have also shown that stearic acid has the potential to prevent and treat breast cancer [20].

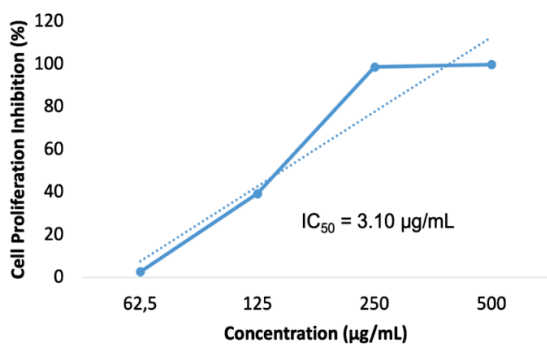
2-hexenoic acid or palmitic acid have been shown to have antitumor activity in mouse models and are cytotoxic selective for MOLT-4 leukemia cancer cells because of their interaction with DNA topoisomerase I and their ability to induce apoptosis [21]. 2-hexenoic acid is also able to inhibit growth and induce apoptosis of human gastric cancer cells [22]. 2-hexenoic acid has been found to have anti-inflammatory, antioxidant, hypocholesterolemic, 5- $\alpha$  reductase inhibitors, nematicide, pesticides and antiandrogenic [23]. The result of MTT assays was obtained from the linear regression equation between concentration versus percent of living cells. The results showed in Figure 4 that 2-hexenoic acid significantly inhibited the MCF-7 cell line and had potent extract with IC<sub>50</sub> value about 3.10  $\mu\text{g mL}^{-1}$ . According to The U.S. The National Cancer Institute (NCI) a compound has cytotoxic activity if it has IC<sub>50</sub> values < 20  $\mu\text{g mL}^{-1}$  [24, 25]. Based on the IC<sub>50</sub> values of the two isolated compounds, both isolates were confirmed the IC<sub>50</sub> values < 20  $\mu\text{g mL}^{-1}$ .

Fatty acids compounds in herbal plants have a role as chemopreventive agents or cause cell cycle inhibition and trigger apoptosis in cancer cells [26]. The results of this study revealed that a lower IC<sub>50</sub> value indicates a higher anticancer activity. This study shows that the fatty acids from octadecanoic acid and hexadecanoic acid are responsible for their pharmacological activity and their extract produces a chemopreventive agent effect or causes inhibition of the growth cycle of cancer cells [27]. The rodent tuber mutant plants showed a potential source that can be used as an alternative treatment for breast cancer from natural ingredients. In the previous study, Purwaningsih et al. [28] conducted cytotoxic tests from the leaves of rodent tuber extracts against HeLa and MCF-7 cells produced IC<sub>50</sub> values of 30.19  $\mu\text{g mL}^{-1}$ , 5.58  $\mu\text{g mL}^{-1}$  respectively, whereas in the study of Purwaningsih et al. [29, 30] obtained by rodent tuber extract can reduce telomerase expression in HeLa and raji cells.



**Fig. 3.** In vitro cytotoxic activity of the isolated compounds 2-octenoic acid (1) in MCF-7 cancer cell lines.

The cytotoxic effects are expressed in terms of the percentage of cell proliferation inhibition after 24 h exposure to the isolated compounds **2-octenoic acid (1)**.



**Fig. 4.** In vitro cytotoxic activity of the isolated compounds 2-hexenoic acid (2) in MCF-7 cancer cell lines.

The cytotoxic effects are expressed in terms of the percentage of cell proliferation inhibition after 24 h exposure to the isolated compounds **2-hexenoic acid (2)**.

## 4 Conclusion

The result showed that the isolated compounds from Tipobio variety were found two fatty acid derivatives, 2-octenoic acid (1) and 2-hexenoic acid (2). Tipobio variety concluded that has an important role and offers a new potential promising as anticancer agents on breast cancer cell. 2-octenoic acid and 2-hexenoic acid (palmitic acid) are bioactive compounds as anticancer. The cytotoxicity test via MTT on MCF-7 cancer cells obtained by 2-octenoic acid and 2-hexenoic acid has high  $IC_{50}$  values of  $2.66 \mu\text{g mL}^{-1}$  and  $3.10 \mu\text{g mL}^{-1}$ , respectively. The further study is needed to investigate the effect of 2-octenoic acid and 2-hexenoic acid on activity apoptosis and telomerase in MCF-7 breast cancer cell lines.

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