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Research Article

IN VITRO CYTOTOXIC ACTIVITY OF RODENT TUBER MUTANT PLANT (*TYPHONIUM FLAGELLIFORME* LODD.) AGAINST TO MCF-7 BREAST CANCER CELL LINE

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ABSTRACT

Objective: The objective of this study was to determine the new bioactive compounds through gas chromatography–mass spectrometry analysis and the cytotoxic activity of two rodent tuber mutant plants against breast cancer cells (MCF-7).

Methods: The bioactive compounds in rodent tuber mutant plants were successfully increased by somaclonal variation using gamma rays irradiation technique. Further, the cytotoxicity activity of rodent tuber mutant plants was tested on breast cancer cell line (MCF-7) performed by 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide assay method.

Results: This results study confirmed that the presence of phytochemical composition in the tuber of rodent tuber mutant plants KB 6–1–3–4 and KB 6–9–5 was found six bioactive compounds from fatty acid groups which have the potential as an anticancer compound, such as octadecanoic acid, hexadecanoic acid, hexadecanoic acid methyl ester, 9-octadecanoic acid, linolelaidic acid methyl ester, and butanoic acid. The results showed that extracts from rodent tuber mutant plants had a cytotoxicity effect on MCF-7 cancer cells with half maximal inhibitory concentration (IC_{50}) values that were lower than the control (mother plant). *In vitro* tests of KB 6–1–3–4 and KB 6–9–5 against MCF-7 cancer cell lines have IC_{50} values of 12.482 µg/mL and 7.043 µg/mL, respectively, while it had a lower cytotoxicity effect with the IC_{50} value of control plant was 19.113 µg/mL. The mutant plants of KB 6-9-5 have 3 times more effective than control.

Conclusion: The results of this study clearly indicated that rodent tuber mutant plants have shown promising as an anticancer drug on breast cancer.

Keywords: *Typhonium flagelliforme* Lodd., Anticancer, MCF-7 cell, Gas chromatography–mass spectrometry analysis, Fatty acids.

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INTRODUCTION

Rodent tuber (*Typhonium flagelliforme* Lodd.) is the Araceae family and classified as herbal plants [1]. Plant propagation methods are carried out vegetatively by separating the shoots [1]. Many studies investigated that rodent tuber has bioactive compounds as anticancer, antiviral, antibacterial, and antioxidant agents [2-4]. Rodent tuber was found to possess useful anticancer activities such as in breast, intestine, prostate gland, liver, leukemia, and cervix [2,5,6]. *In vitro* mutagenesis was carried out using a combination of gamma-ray irradiation and somaclonal variation to increase the bioactive compounds in rodent tuber plants. The rodent tuber mutant plants have morphology changes [7], genetic changes [8,9], and protein expression changes [10]. Morphological, genetic, and protein expression changes can reinforce the bioactive compounds in mutant plants. Sianipar and Purnamaningsih [11] had investigated that bioactive compounds in several mutant clones from Bogor have been increased.

Genetic mutations can be used and the number of phytochemical components in plants, which can be detected by gas chromatography–mass spectrometry (GC–MS). GC–MS has been used to determine the content of herbal plants such as *Melia orientalis* [12], *Maranta arundinacea* L. [13], and non-polar fractions of Malaysian rodent tuber [6]. Extracts of the rodent tuber plant have been applied to several cancer cells *in vitro*. Rodent tuber extract with ethanol fraction has been shown to be effective in inhibiting the growth of T47D breast cancer cells [14], inhibiting the proliferation of T4-lymphoblastoid human cancer cells [4,5] and can inhibit the growth of cell cultures of non-small cell lung carcinoma NCI-H23 [2]. The anticancer activity test can be carried out by antiproliferative extract test on the growth of cancer cells using the clonogenic assay method [15,16]. The 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method has been used to determine anticancer activity in rodent tuber [2,4,14]. The aimed of this study was to determine the new bioactive compounds through GC–MS analysis and has a cytotoxic activity of two mutant clones (KB 6–1–3–4 and KB 6–9–5) against MCF-7 breast cancer cell lines.

METHODS

Plant material

Rodent tuber plant material is used from Bogor. Rodent tuber has been irradiated by gamma-ray to produce *in vitro* mutagenesis and obtained rodent tuber mutant plants. Two mutant plants of KB 6-1-3-4, KB 6-9-5, and control plant (mother plant) were acclimatized and maintained at Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Ministry of Agriculture, Bogor, Indonesia. The rodent tuber mutant plants were harvested and dried to extract all substances.

Preparation of rodent tuber mutant extract

Rodent tuber mutant plants KB 6-1-3-4, KB 6-9-5, and control plants were harvested and cleaned the tubers, then cut into small pieces and weighed. The tuber samples were macerated using ethanol for 24 h, 2 times repetition [17]. The samples were filtered using Whatman paper No.1, and the filtrate was evaporated using a rotary evaporator at 40°C to obtain a crude extract of rodent tuber. Extraction results are put into a sample bottle and dried using freeze drier.

GC–MS analysis

The ethanol fraction of the sample was injected into the GC column. The injection volume of the extraction solution was 5 µl with a 5:1 split ratio and an injection temperature of 250°C. Helium is used as a carrier gas with a speed of 0.8 µl per min. The column temperature was set at 70°C with an increase of 50°C per min. When the temperature reaches 200°C, it remains constant for 1 min and then increases at a rate of 20°C/min until the temperature reaches 280°C. The temperature remains constant for 28 min. The mass spectrometer was operated in electron ionization mode with a voltage of 70 eV.

Identification of phytochemical compounds

GC–MS mass spectrum identification was carried out with reference to the National Institute of Standards and Technique database with a factor of fit ≥90% [18]. Percentage of the relative abundance of each compound was calculated by comparing the average peak area with the total area.

***In vitro* screening test of cancer cell cytotoxicity and data analysis**

Cytotoxic activity against breast cancer cells (MCF-7) performed by MTT assay method. 100 µL of suspension 10^3 cells were cultured and seeded into 96-well disk plate, and incubated at 37°C for 24 h. 100 µL of medium containing rodent tuber extract with serial concentration obtained 1000, 500, 250, 125, and 62.5 µg/mL of test solution added to each well and the dish was incubated at 37°C for 24 h. At the end of the incubation, the culture media containing the sample was removed and washed with 100 µL phosphate-buffered saline. In each single well was added 100 µL of culture medium containing MTT and reincubated for 4 h at 370°C. The live cells will react with MTT to form purple formazan. After 4 h, at each well, the stopper reagent was added to kill cells and dissolved formazan crystals. Plate in a shaker for 10 min, then incubated at room temperature in a dark room overnight. Furthermore, the absorbance of each well was read by the ELISA reader with absorbance at a wavelength of 595 nm, and the percentage of cell viability of MCF-7 or cell proliferation inhibition percentage was calculated. A dose of test solution that reduces survival by half maximal inhibitory concentration (IC_{50}) was calculated. Cisplatin was used as a positive control while control plant was used as a negative control. The cytotoxic activity of rodent tuber mutant plants was expressed with IC_{50} values obtained through a linear regression analysis between the logarithm of the concentration of the test material and the percentage of MCF-7 cell viability.

RESULTS

The bioactive compounds in the tuber of mutant clones extract have been identified using GC–MS analysis. Based on the GC–MS analysis, there were differences in the phytochemical composition between the tuber of control and mutant plants (Table 1). This study evaluated that the phytochemical composition in the tubers of KB 6–1–3–4 and KB 6–9–5 mutant clones was increased and found new bioactive compounds which not present in the control plant (Table 1). The tubers of mutant clones contained several new anticancer compounds which were not found in control (Table 2 and Fig. 1).

The results of this study revealed that six bioactive compounds from fatty acid have potential as anticancer activity is octadecanoic acid, hexadecanoic acid, hexadecanoic acid methyl ester, 9-octadecanoic acid, linolelaidic acid methyl ester, and butanoic acid (Table 2). Some anticancer compounds in mutant clones have a higher amount than control plant. The most potential target as anticancer properties among six bioactive compounds from fatty acid is octadecanoic acid and hexadecanoic acid. Octadecanoic acid has the most different anticancer properties with the relative abundance. The octadecanoic acid was absent in the control plant. As shown in Table 2, octadecanoic acid in the KB 6-9-5 has higher concentrations of about 85.03%. This study confirmed that KB 6-9-5 has potency as anticancer agent 2 times greater than KB 6-1-3-4 mutant clones.

Table 1: Phytochemical composition in the tubers extraction of rodent tuber control and mutant clones based on GC–MS

Name of compound	RT			Area percentage		
	Control	6-1-3-4	6-9-5	Control	6-1-3-4	6-9-5
Hexadecanoic acid	31.954	28.889	28.861	22.08	4.46	3.99
Hexadecanoic acid, methyl ester	NI	28.331	NI	NI	2.86	NI
Octadecanoic acid	NI	89	30.144	NI	8.07	85.03
9-Octadecanoic acid, methyl ester	NI	29.517	NI	NI	2.39	NI
9,12-Octadecadienoic acid	33.078	NI	NI	38.37	NI	NI
Campesterol	40.532	NI	NI	1.55	NI	NI
Alpha-monopalmitin	NI	NI	38.439	NI	NI	1.07
Stigmasterol	40.966	NI	NI	4.05	NI	NI
Butanoic acid	NI	28.820	NI	NI	2.53	NI
2-Acetyl-2-thiazoline	NI	NI	29.510	NI	NI	1.09
Linolelaidic acid, methyl ester	NI	29.489	NI	NI	6.09	NI
N-Acetyl-d, 1-norleucenine	NI	29.648	NI	NI	2.23	NI
11-Dodecenyl trifluoroacetate	NI	29.985	NI	NI	47.64	NI
1-Acetoxynonane	NI	31.571	NI	NI	2.72	NI
(Z, Z)-3,6-Nonadienal	NI	32.578	NI	NI	7.28	NI
1-Tridecene	NI	37.970	NI	NI	2.90	NI
1,5-Heptadiene, 3,3,5-trimethyl	NI	38.543	NI	NI	4.93	NI

Table 2: Fatty acid composition as anticancer properties in the tubers extraction of rodent tuber mutant clones

Anticancer properties reference	Name of the compound	Nature of the compound	RT		Area percentage	
			6-1-3-4	6-9-5	6-1-3-4	6-9-5
[19,20]	Hexadecanoic acid	Palmitic acid	28.889	28.861	4.46	3.99
[21]	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	28.331	NI	2.86	NI
[19,22]	Octadecanoic acid	Stearic acid	89	30.144	8.07	85.03
[19,23]	9-Octadecanoic acid, methyl ester	Omega-9/elaidic acid	29.517	NI	2.39	NI
[2]	Linolelaidic acid, methyl ester	Omega-6	29.489	NI	6.09	NI
[25-27]	Butanoic acid	Butyric acid	28.820	NI	2.53	NI

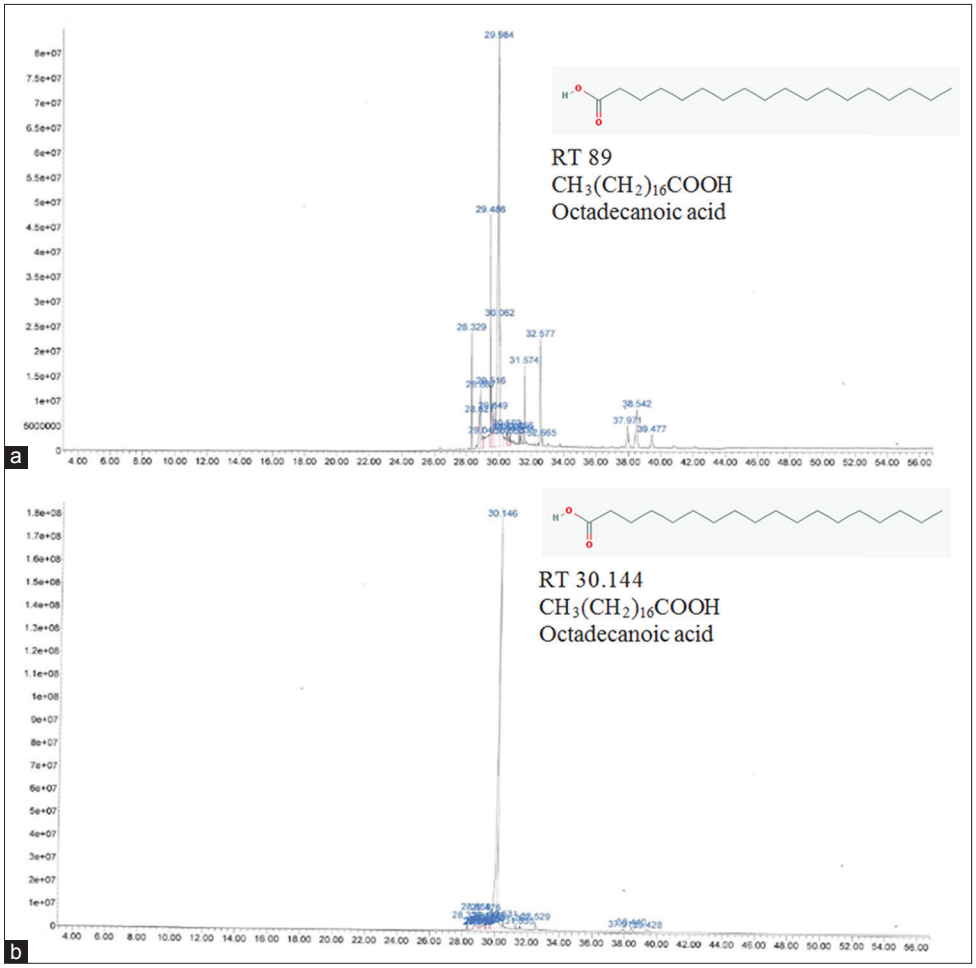

Fig. 1: Gas chromatography–mass spectrometry analysis of the ethanol extract of tuber (a) KB 6-1-3-4 and (b) KB 6-9-5

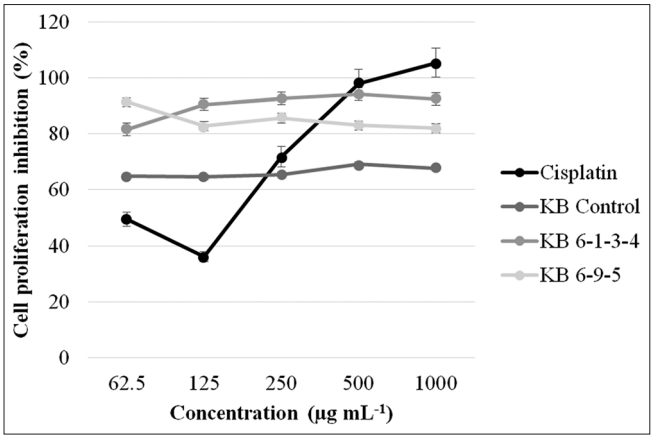

Fig 2: The graph represents the cell proliferation inhibition of tuber mutant plants (KB 6–1–3–4 and KB 6–9–5) against MCF-7 breast cancer cell lines at different concentrations (62.5–1000 µg mL^{-1}). Data were plotted as a percentage of cell proliferation inhibition and represented as mean ± standard error mean (SEM) of three independent experiments

The result of MTT assays was obtained from the linear regression equation between concentration and percent of living cells. The results showed that the tubers extract from two mutant clones significantly inhibited the MCF-7 cell line and had potent extract with IC_{50} value of 12.482 µg/mL from KB 6-1-3-4 and 7.043 µg/mL from KB 6-9-5. All the tubers extract from two mutant clones showed cytotoxic effect toward MCF-7 cell line.

According to the U.S. The National Cancer Institute (NCI), a compound has cytotoxic activity if it has IC_{50} values of <20 µg/mL [28,29]. Based on the IC_{50} values of the two rodent tuber mutant plants, the IC_{50} values of <20 µg/mL so that it can be confirmed that both KB 6–1–3–4 and KB 6–9–5 have cytotoxic activity against MCF-7 cell line. The results of this study showed a linear relationship between IC_{50} values of KB 6–1–3–4 and KB 6–9–5 with a percentage of viability of living cells (Fig. 2). The IC_{50} value of the control rodent tuber plant was 19.1131 µg/mL. The result of MTT assay on rodent tuber mutant plant's extract KB 6-9-5 was 3 times more effective than compared to control plant.

DISCUSSION

Octadecanoic acid or stearic acid is a saturated fatty acid found in relatively high concentrations in some foods. Octadecanoic acid or stearic acid is the primary metabolites present in plants which form of glycerol esters [30]. Stearic acid has been reported to inhibit the development of human breast cancer cells in proliferation *in vitro* [31-33] and *in vivo* [34]. Stearic acid has also been shown to induce apoptosis in breast cancer cells and inhibit cell cycle of breast tumors [35,36]. Interestingly, epidemiological studies have also shown that stearic acid has the potential to prevent and treat breast cancer [37].

Hexadecanoic acid or palmitic acid has been shown to have antitumor activity in mouse models and is cytotoxic selective for MOLT-4 leukemia cancer cells due to their interaction with DNA topoisomerase I and their ability to induce apoptosis [24]. Palmitic acid was present in *Solanum nigrum* which had a role induce apoptosis on cervical cancer cell lines [38]. The hexadecanoic acid methyl ester is also able to inhibit growth and induce apoptosis of human gastric cancer cells [39]. Hexadecanoic acid methyl ester has been found to have anti-

inflammatory, antioxidant, hypocholesterolemic, 5-alpha reductase inhibitors, nematicide, pesticides, and antiandrogenic [40].

Fatty acid compounds in herbal plants have a role as chemopreventive agents or cause cell cycle inhibition and trigger apoptosis in cancer cells [41]. The results of this study revealed that a lower IC_{50} 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 [42]. 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.* [43] conducted cytotoxic tests from the leaves of rodent tuber extracts against HeLa and MCF-7 cells produced IC_{50} values of 30.19 µg/mL, 5.58 µg/mL, respectively, whereas in the study of Purwaningsih *et al.* [44,45] obtained by rodent tuber extract can reduce telomerase expression in HeLa and Raji cells.

This study showed that KB 6–1–3–4 and KB 6–9–5 extracts have the strong potential as anticancer agents through inhibition of MCF-7 cancer cell. Mohan *et al.* [4] have examined that the cytotoxic effects of the crude extracts of rodent tuber plants using dichloromethane and ethyl acetate on leukemia cancer cells (T4-lymphoblastoid CEM-ss) have a significant effect in inhibiting cell invasion and experiencing apoptosis for 72 h, at IC_{50} values of 6.5 and 8.2 µg/mL. Antitumor and cytotoxic effects have been observed for the chemical compounds methyl esters [46] and other evidence suggests that amino acid methyl esters produce high cytotoxic effects on MCF-7 cells [35]. In addition, as shown in the study of Hardy *et al.* [47], octadecanoic acid induces apoptosis of breast cancer cells through mechanisms that might have implications for increased cardiolipin replacement and a reduction of mitochondrial phospholipids.

CONCLUSION

Mutant clones of KB 6–1–3–4 and KB 6–9–5 concluded that have an important role and offer a new potential promising as anticancer agents on breast cancer cell. Octadecanoic acid (stearic acid) and hexadecanoic acid (palmitic acid) are the major role of bioactive compounds as anticancer. The cytotoxicity test through MTT on MCF-7 cancer cells obtained by KB 6-9-5 mutant clone has higher IC_{50} values of 7.043 µg/mL which compared with the IC_{50} values in control plants about 19.113 µg/mL. KB 6-9-5 mutant clone has greater effectiveness up to 5 times compared to control plants. KB 6–1–3–4 and KB 6–9–5 showed more effective as anticancer against *in vitro* MCF-7 breast cancer cells. The further study is needed to investigate the effect of rodent tuber mutant plants extract on activity apoptosis and telomerase in MCF-7 breast cancer cell lines.

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AUTHORS' CONTRIBUTIONS

All authors declare that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

CONFLICTS OF INTEREST

All authors declare that this material or similar material has not been and will not be submitted to or published in any other publication. There are no any potential conflicts of interest.

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