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Enhancement of the Contents of Anticancer Bioactive Compounds in Mutant Clones of Rodent Tuber (*Typhonium flagelliforme* Lodd.) based on GC-MS Analysis

Nesti Fronika Sianipar[1,2]* and Ragapadmi Purnamaningsih[3]

[1]Department of Food Technology, Faculty of Engineering, Bina Nusantara University, 11480 Jakarta, Indonesia
[2]Research Interest Group Food Biotechnology, Bina Nusantara University, 11480 Jakarta, Indonesia
[3]Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (BB-Biogen), 16111 Bogor, Indonesia

ABSTRACT

Rodent tuber (*Typhonium flagelliforme* Lodd.), which is a well known herbal plant from the Araceae family, is know for its anticancer activities. The genetic variation of rodent tuber is low due to its commonly applied vegetative propagation methods. Thus, its genetic variation has to be increased to obtain a new and superior plant containing a high amount of anticancer compounds. The aim of this study was to analyse the chemical compounds of the leaves and tubers of rodent tuber' mutant and non irradiated (control) plants by GC-MS method. In this study, *in vitro* calli of rodent tuber was irradiated with 6 Gy of gamma ray which produced mutant plantlets which was genetially different from non irradiated (control) plants. Mutant plantlets had been acclimated and propagated in the greenhouse to obtain the 6th generation vegetative mutant clones (MV6), which are stable superior mutants. The results indicated that MV6 contained six new anticancer compounds in the leaves and four new anticancer compounds in the tubers which have not been detected in control. The new anticancer compounds present in leaves and tubers were identified by GC-MS. They are hexadecanoic acid methyl ester, octadecadienoic acid, phytol, gamma-sitosterol, eicosane, geranylgeraniol, squalene, octacosane and 7-pentadecyne. MV6, is the new superior variety and a potential source of anticancer drugs.

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E-mail addresses:
nestipro@yahoo.com;
nsianipar@binus.edu (Nesti Fronika Sianipar),
raga_padmi@yahoo.com (Ragapadmi Purnamaningsih)
* Corresponding author

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INTRODUCTION

Rodent tuber (*Typhonium flagelliforme* Lodd.) is a medicinal herbal plant from Indonesia that belongs to the Araceae family (Essai, 1986). Biologically active chemicals in this plant are alkaloids, saponins, steroids, and glycosides (Syahid, 2007). A study has documented the effectiveness of ethanolic fraction of the rodent tuber' extract in inhibiting the growth of T47D breast cancer cell line (Nurrochmad et al., 2011) while another has show the efficacy of its dichloromethane fraction against MCF-7 breast cancer cell line (Putra & Winarto, 2012). The rodent tuber's extract has also been found toinhibit the proliferation of human T4 lymphoblastoid (Mohan et al., 2008) and NCI-H23 non-small lung carcinoma cell line (Lai, et al., 2008). Anticancer compounds could be found in all parts of the plant, such asthe roots, tubers, stems, and leaves (Choo et al., 2001). The hexane extract of rodent tuber was proven to be cytotoxic against *Artemia salina* larvae (Sianipar et al., 2013a). This plant also has antibacterial and antioxidant properties (Mohan et al., 2008).

The main obstacle in the development of rodent tuber into drugs are its low genetic diversity and low organic compounds content (Syahid, 2008). The low genetic variation is due to commonly practiced vegetative propagation methods, mainly through conventional buds separation (Essai, 1986). Although vegetative propagation methods could produce seedlings, these methods rarely cause genetic recombination. Thus, the genetic variation in species level is low and reduce the creation of new genotypes (Syahid & Kristina, 2007). Genetic variation of rodent tuber has to be increased in order to obtain superior mutant clones which contain a high amount of anticancer compounds. Mutation induction is an effective way to its increase genetic diversity (Wulan, 2007). The mutation could be induced by irradiating the respective sample with physical mutagens, such as gamma ray (Poespordasono, 1988). *In vitro* embryogenic somatic cell population or calli of rodent tuber has been induced, proliferated, and regenerated with single node culture method (Sianipar et al., 2011).

In an earlier study, the rodent tuber calli were irradiated with gamma ray. They were regenerated into in vitro plantlets that showed various growth responses based on the observation of the number of shoots, number of leaves, and plant's height (Sianipar et al., 2013b). Rodent tuber calli was irradiated at many doses to induce mutation. Rodent tuber mutation induction successfully done at the dose 6 Gy. The 1st generation plantlet of rodent tuber through gamma rays irradiation can detect genetic changes of mutant by using RAPD (Sianipar et al., 2015b).

In the first generation of vegetative mutant (MV1 generation) 37 mutant clones were discovered which had several different morphological characteristics from control plants (Sianipar et al., 2013c). Out of those, 17 clones had genetic differences with control plants according to PCR-RAPD molecular marker analysis (Sianipar et al., 2015a). These 17 mutant clones were

propagated and regenerated into the 6th generation of vegetative mutant (MV6).

Extraction involves isolation or purification of chemicals from raw samples (Mohrig, 2010). Mutant clones contain a lot of bioactive compounds with various functional groups and polarities (Hota, 2007). Hydrophilic substances were extracted with polar solvents, such as ethanol (Rostagno et al., 2013). Genetic mutation could affect the relative abundances of plant's bioactive compounds. Gas Chromatography-Mass Spectrometry (GC-MS) is able to analyse the metabolomic profile of an organism. GC uses gas as its mobile phase to separate various chemicals. The MS could separate chemicals based on their molecular weight (Kayser & Quax, 2007). The GC-MS is a powerful device to identify chemicals by referring to their databases (Kayser & Quax, 2007) and it has been applied to analyse phytochemical and bioactive compounds of herbal plants, such as *Melia orientalis* (Marimuthu, 2013) and *Maranta arudinacea* L. (Nishaa, 2013). The GC-MS has also been employed to identify chemical compounds in the nonpolar fraction of rodent tuber from Malaysia (Mohan et al., 2011). The aim of this study is to analyse the bioactive anticancer compound content in the polar fraction of rodent tuber stable superior mutant clones by using GC-MS.

MATERIALS AND METHODS

Plant material

The 1st generation of mutant plantlets irradiated by gamma rays (MV1) were acclimatised and propagated in the greenhouse to obtain the 6th generation of vegetative mutant clones (MV6). The 6th generation of vegetative stable superior mutant population (MV6) from Bogor (Sianipar et al., 2013), is in the patenting process. There were 8 mutant MV6 clones (6-3-3-6, 6-1-1-2, 6-3-2-5, 6-9-1, 6-2-5-2, 6-1-2, 6-9-4, 6-2-6-3) and 1 (non-irradiated) for control. Each sample had 9 replicates. The leaves and tubers of rodent tubers underwent metabolite extraction.

Preparation of extract from rodent tuber

The rodent tuber was dried and macerated in 96% ethanol overnight. The solvent was removed after it was filtered through Whatman filter paper No. 1. The concentrated extract was collected and used for GC-MS analysis.

Identification of chemical content with GC-MS

The concentrated ethanol extracts were injected into the GC column. Injection volume was 5µl with 5:1 split ratio and the injection temperature was 250°C. Helium was used as carrier gas with velocity 0.8 µl per minute. Column temperature was set at 70°C with 5°C per minute increment. At 200°C, the temperature was kept constant for 1 minute before it was increased to 280°C at the rate of 20°C per minute. The temperature remained constant for another 28 minutes. Mass spectrometer wasused in electron impact ionisation mode with 70 eV voltage.

The mass spectrum of GC-MS was identified by referring to the National Institute Standard Technique (NIST) database with ≥ 90% fit factor. The content of the compound was calculated by comparing its average peak area to the total area.

RESULTS AND DISCUSSION

The results of fresh and dry weight for each clone (Table 1) are different. Mutant clones 6-1-2, 6-9-4, 6-2-6-3, and 6-2-5-2 had higher stem, leaves and tuber weight than control. Mutant clone 6-2-5-2 had the highest fresh and dry weight compared with control. The highest tuber dry weight was obtained by clones 6-1-2 and 6-2-5-2. The differences in fresh and dry weight are due to genetic changes or mutation, but the increase in fresh or dry weight did not occur in all mutant clones. Genetic changes are not always followed by morphological changes. Sianipar et al. (2013) have shown irradiated calli can lead to genetic changes. These genetic changes are evidenced by genetic variation in 1st generation mutant of the rodent tuber (MV1), 3rd generation mutant (MV3), 4th generation mutant (MV4) by using RAPD (Sianipar et al., 2015a; 2016; 2017).

In vitro culture treatment and gamma ray irradiation could induce chromosomal abberation i.e. the modification of chromosomal number and structure (Surya & Soeranto, 2006; Pillay & Tenkouano, 2011). These modifications can alter morpho-physiological properties of mutant clones (Table 1). The DNA mutation will lead to the generation of new genotypes and affect transcription, translation, protein synthesis and enzyme expressions that leads to production of secondary metabolites (Gorbunova & Levy, 1997; Kovacs & Keresztes, 2002).

Table 1
Fresh and dry weights of rodent tuber's control and mutant clone

Plant	Total fresh weight (g)	Dry weight (g)		
		Total	Leave and stem	Tuber
Field control	150	38,8	8,7	32
In vitro control	100	22,7	NM	19
6-9-1	100	17,3	7,4	13
6-3-3-6	100	26	8,4	20
6-3-2-5	50	20,1	2,5	20
6-1-1-2	50	23	5,7	21
6-1-2	162,5	34,48	14	37
6-9-3	50	17,4	NM	NM
6-9-4	100	38,2	8,9	30
6-1-3-4	50	22	NM	NM
6-2-6-3	200	34,5	13,5	29
6-2-4-1	150	22	NM	NM
6-2-5-2	300	54,8	17,9	37

Note: NM stands for 'Not Measured'

Chemical composition of the leaves, tubers of rodent tuber's control and mutant plants were successfully identified with GC-MS (Figure 1, Tables 2-5), which showed that there were phytochemical profile differences between control and mutant clones as well as between each of the mutant clones. Leaves and tubers of control plants contained 19 and 26 different chemicals respectively (Tables 2 and 3). Five most abundant chemicals in the leaves of control plant were 9,12,15-octadecatrienoic acid or linolenic acid, hexadecanoic acid, stigmasterol, (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol, and campesterol (ergost-5-en-3-ol). Meanwhile, five most abundant compounds in the tubers of control plant were (9E,12E)-9,12-octadecadienoic acid, hexadecanoic acid/palmitic acid, methyl (9z,12z)-9,12-octadecadienoate, stigmasterol and ergost-5-en-3-ol.

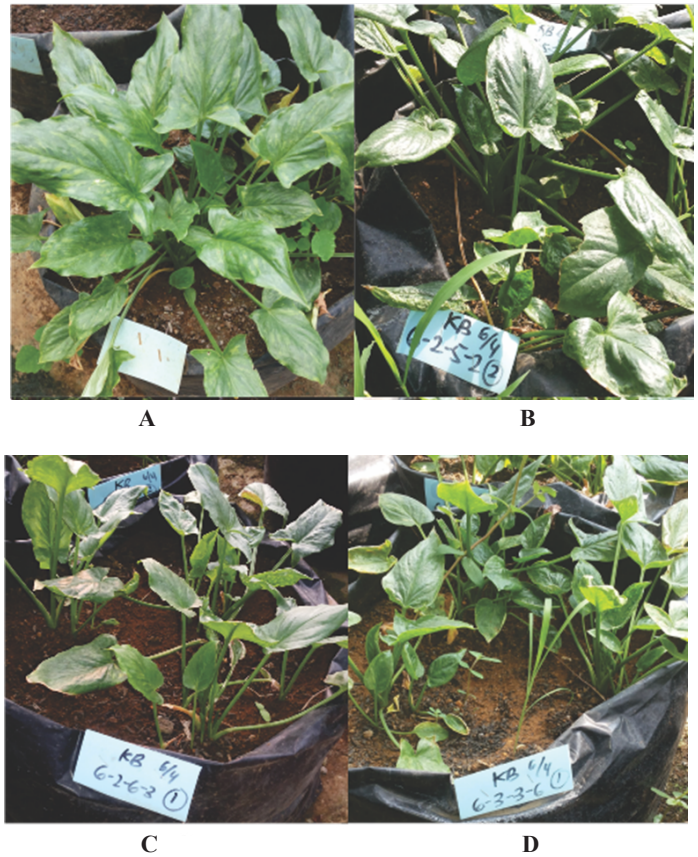

Figure 1. Control and stable superior mutant clones of rodent tuber at 8th week in greenhouse
Note: A = Control; B = 6-2-5-2; C = 6-2-6-3; D = 6-3-3-6

Table 2
Chemical compounds in leaves of rodent tuber control plant based on GC-MS

RT	Relative abundance (%)	Chemical compound
30,051	2,98	2,6,10-trimethyl,14-ethylene-14, pentadecne (neophytadiene)
30,34	0,94	2,6,10-trimethyl,14-ethylene-14, pentadecne (neophytadiene)
30,534	1,24	2,6,10-trimethyl,14-ethylene-14,pentadecne (neophytadiene)
31,54	0,2	Hexadecanoic acid, ethyl ester
31,678	20,77	Hexadecanoic acid
32,216	0,36	9,17-octadecadienal
32,257	0,86	9,12,15-octadecatrienoic acid, methyl ester (linolenic acid methyl ester)
32,375	5,87	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol
32,602	1,05	Ethyl (9z,12z)-9,12-octadecadienoate
32,795	41,16	9,12,15-octadecatrienoic acid (linolenic acid)
33,278	0,9	Methyl 8,11,14-heptadecatrienoate
35,043	0,12	Oleic acid (9-octadecadienoic acid)
35,746	0,6	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl (squalene; spinacene)
36,146	0,36	Octacosane
39,587	3,16	Campesterol (ergost-5-en-3-ol)
39,939	7,67	Stigmasterol
40,746	1,96	Beta-sitosterol
44,8	2,87	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-
50,158	0,25	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-

Note: Compounds were identified by comparing retention time data with authentic standard database of NIST/EPA/NIH fit factor ≥ 90%. Relative abundance was determined based on area percentage of each compound

Table 3
Chemical compounds in tubers of rodent tuber control plant based on GC-MS

RT	Relative abundance (%)	Chemical compound
31,168	0,08	Farnesol, methyl ether
31,954	22,08	Hexadecanoic acid (palmitic acid)
32,526	0,73	Hexadecanoic acid (palmitic acid)
32,581	0,22	Hexadecanoic acid (palmitic acid)
32,616	0,26	Heptadecanoic acid (potassium heptadecanoate)
32,699	0,2	n-hexadecanoic acid (palmitic acid)
32,919	0,85	linoleic acid ethyl ester (ethyl linoleate)
33,078	38,37	(9E,12E)-9,12-octadecadienoic acid
33,616	4,34	Methyl (9z,12z)-9,12-octadecadienoate
33,85	1,15	(9E,12E)-9,12-octadecadienoic acid

Table 3 *(continue)*

33,953	0,89	4-(4-ethylcyclohexyl)-1 pentyl-1-cyclohexene
34,036	1,27	2-aminoethanol hydrogen sulfate (ester)
34,36	1,08	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-
34,422	1,05	z,e-3,13-octadecadien-1-ol acetate (9e,12e)-9,12-octadecadienoic acid
34,795	1,24	9,12-octadecadienoic acid (grapeseed oil)
36,084	0,73	farnesol isomer A
36,656	0,34	Eicosane (Icosane)
36,994	0,25	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-
37,705	0,19	Stigmast-5-en-3-ol
38,753	1,78	Peri-xanthenoxanthene-4,10-dione, 2,8-bis (1-methylethyl)-
39966	0,61	Solanesol
40,532	1,55	campesterol (ergost-5-en-3-ol)
40,573	2,38	Ergost-5-en-3-ol
40,966	4,05	Stigmasterol
41,876	1,92	Gamma-sitosterol
41,945	1,63	Stigmast-5-en-3-ol

Note: Compounds were identified by comparing retention time data with authentic standard database of NIST/EPA/NIH fit factor ≥ 90%. Relative abundance was determined based on area percentage of each compound

One of the clones with highest amount of anticancer compounds was 6-1-2 (Tables 4 and 5). Either in the leaves or tubers of this clone, the most abundant compound was 9,12-octadecadienoic acid (Figure 2). Octadecadienoic acid could induce apoptosis of various cancer cells (Yoo et al., 2007). The other most abundant compounds in leaves of 6-1-2 were 9,12-octadecadienoic acid, hexadecanoic acid, phytol, neophytadiene, and stigmasterol. While in its tubers were (9E,12E)-9,12-octadecadienoic acid, hexadecanoic acid, hexadecanoic acid ethyl ester, and ethyl (9Z,12Z)-9,12-octadecadienoate.

The amount of some anticancer bioactive compounds in mutant clones were higher than control. Mutant clones also contained several new anticancer compounds which were not found in control (Tables 6 and 7). Leaves of mutant clones had at least six anticancer compounds and in greater quantities compared with control. The amount of hexadecanoic acid was the highest in mutant clone 6-9-4, about 15.04% compared with control. Also known as palmitic acid, it has cytotoxic effect against MOLT-4 leukimia (cancer cell line) by interacting with DNA topoisomerase I to induce apoptosis (Kwan et al., 2014). Palmitic acid has also been reported to exhibit antitumour activity in vivo (Harada et al., 2002).

Table 4
Chemical compounds in leaves of rodent tuber mutant clone 6-1-2 based on GC-MS

RT	Relative abundance (%)	Chemical compound
30,147	5,43	2,6,10-trimethyl,14-ethylene-14, pentadecne (neophytadiene)
30,416	1,04	2,6,10-trimethyl,14-ethylene-14,pentadecne (neophytadiene)
30,602	1,89	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol
31,051	0,23	Hexadecanoic acid, methyl ester
31,588	0,13	Hexadecanoic acid, ethyl ester
32,023	14,44	Hexadecanoic acid
32,278	0,94	Hexadecanoic acid
32,319	1,62	Hexadecanoic acid
32,478	6,73	Phytol
32,678	1,03	9,12-octadecadienoic acid, ethyl ester
33,085	36,01	9,12-octadecadienoic acid
33,354	1,96	13-tetradece-11-yn-1-ol
33,443	1,84	Methyl 8,11,14-heptadecatrienoate
33,692	1,01	(9E, 12E)-9,12-Octadecadienoic acid
34,588	0,16	8-(2-octylcyclopropyl)octanal
36,263	0,27	Nonacosane
39,966	3,7	Ergost-5-en-3-ol
40,387	3,71	Stigmasterol
40,408	4,68	Stigmasterol
41,249	4,32	Cholest-5-en-3-ol,23-ethyl-, (3 beta 23s)
45,489	2,97	5,5-dimethyl-7,8-epoxyspiro (3.5) nonan-1-one
51,737	0,03	5,9-dimethyl-4,10-octadecadiene

Note: Compounds were identified by comparing retention time data with authentic standard database of NIST/ EPA/NIH fit factor ≥ 90%. Relative abundance was determined based on area percentage of each compound

Table 5
Chemical compounds in tubers of rodent tuber mutant clone 6-1-2 based on GC-MS

RT	Relative abundance (%)	Chemical compound
31,912	8,26	Hexadecanoic acid, ethyl ester
31,968	22,8	Hexadecanoic acid
32,568	1,41	9,12-octadecadienoic acid, methyl ester (linoleic acid, methyl ester/ methyl linoleate)
32,954	6,67	Ethyl (9Z,12Z)-9,12-octadecadienoate
33,03	24,29	9,12-octadecadienoic acid
33,112	23,4	(9E,12E)-9,12-octadecadienoic acid
33,616	1,4	Tricosane
34,064	0,83	Z,Z-10,12-hexadecadien-1-ol acetate
34,147	1,84	9,12-octadecadienoic acid

Table 5 *(continue)*

34,505	0,56	Eicosane
35,464	0,89	Eicosane
36,263	1,05	(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene
36,705	1,71	Heptacosane, 1-chloro
38,456	0,83	Nonacosane
41,063	1,03	Stigmasterol
41,152	0,04	Stigmasterol

Note: Compounds were identified by comparing retention time data with authentic standard database of NIST/EPA/NIH fit factor ≥ 90%. Relative abundance was determined based on area percentage of each compound

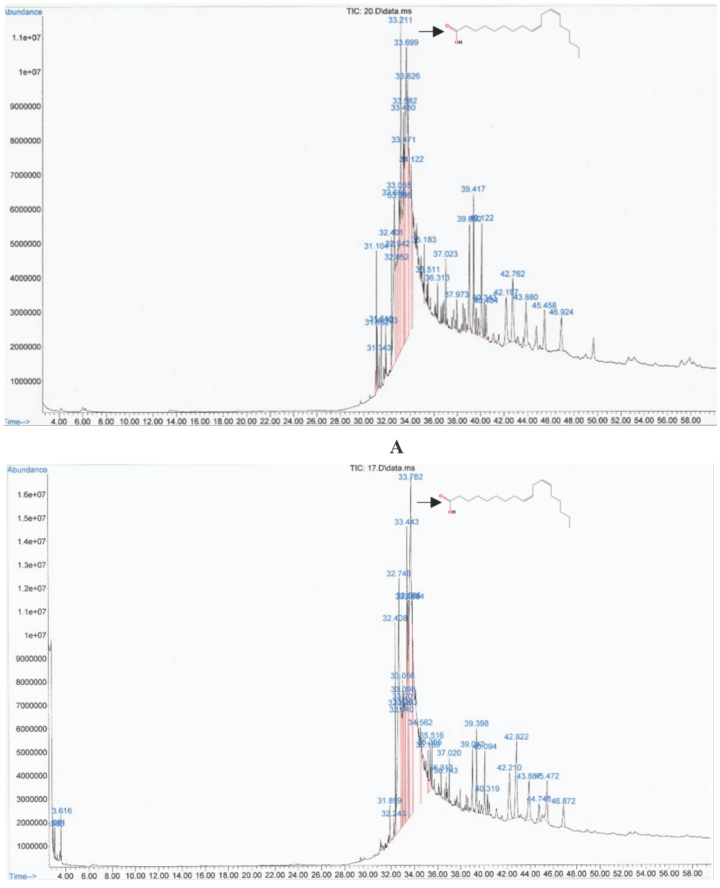

Figure 2. GC-MS chromatogram of leaves and tubers of mutant clones. X-axis represents retention time while Y-axis represents relative abundance. The chemical structure of 9,12-octadecadienoic acid, which has the highest relative abundances are shown (chemical structure obtained from NIST database) here
Note: A = leaves of 6-1-2, B = tubers of 6-1-2

Table 6
Comparison of the relative abundances of anticancer compounds in the rodent tuber's control and mutant plants based on GC-MS analysis

	Name of compound	Relative abundance (%)								
		Control	6-3-3-6	6-1-1-2	6-3-2-5	6-9-1	6-2-5-2	6-1-2	6-9-4	6-2-6-3
1.	Hexadecanoic acid ethyl ester	0,2	0,41	0,91	1,43	0,52	0,52	0,13	0,93	0,84
2.	Hexadecanoic acid methyl ester	NA	0,46	0,36	NA	0,29	NA	0,23	NA	NA
3.	Hexadecanoic acid	20,77	25,35	23,91	22,26	19,87	17,81	17	35,81	18,79
4.	Octadecadienoic acid	NA	0,22	1,4	NA	0,98	NA	37,02	NA	NA
5	Squalene	0,6	1,79	1,21	2,34	1,46	0,98	NA	1,77	1,04
6.	Campesterol (ergost-5-en-3-ol)	3,16	0,21	NA	0,71	1,93	3,42	3,7	NA	3,93
7.	Stigmasta-5,22-dien-3-ol (3 beta) (stigmasterol)	7,67	0,35	6,48	4,48	5,49	8,69	8,39	8,74	9,63
8.	Stigmast-5-en-3-ol (beta-sitosterol)	1,96	0,11	0,97	1,28	NA	NA	NA	NA	NA
9.	Phytol isomer	NA	NA	5,41	NA	NA	9,77	6,73	NA	8,78
10.	Gamma sitosterol	NA	NA	0,68	NA	1,63	NA	NA	NA	NA
11.	Pyridine-3-carboxamide,oxime	3,12	NA	0,41	NA	0,73	6,38	NA	NA	2,04
12.	Eicosane (Icosane)	NA	NA	1,61	NA	NA	NA	NA	NA	NA
13.	Geranylgeraniol	NA	NA	NA	0,63	NA	NA	NA	NA	NA

Note: Chemicals were identified by comparing the retention time with authentic standard database of NIST/EPA/NIH (fit factor ≥ 90%). NA is not available, which means that the quantity of the compound is too low to be detected by GC-MS. The quantities of anticancer bioactive compounds in MV6 mutant clones which are higher than control are indicated by the grey highlights

Leaves of mutant clones contained six new anticancer compounds, namely hexadecanoic acid methyl ester, octadecadienoic acid, phytol, gamma-sitosterol, eicosane, and geranylgeraniol. Hexadecanoic acid methyl ester has been known to to inhibit the growth and induce apoptosis of human gastric cancer cells (Yu et al., 2005). Phytol is an antitumour chemical which could induce the apoptosis of human gastric adenocarcinoma (Song & Cho, 2015) and hepatocellular carcinoma cells. Therefore, it has good potential as a medicine for liver cancer (Kim et al., 2015). Additionally, phytol is cytotoxic against the MCF-7 breast adenocarcinoma cells, but it did not harm normal cells in humans (Peijin et al., 2014).

Gamma-sitosterol has anticancer activity against MCF-7 breast cancer cell and A549 lung cancer cell lines by inhibiting their growth, stopping the cell cycle, and inducing apoptosis (Peijin et al., 2014). Eicosane is a derivative of methyl ester which could inhibit the growth of SGC-7901 gastric cancer cell (Yu et al., 2005). Geranylgeraniol can lead to fragmentation of the DNA of HL-60 leukemia and inhibit the proliferation of DLD1 colon

Table 7
Comparison of the relative abundances of anticancer compounds in the rodent tuber's control and mutant plants based on GC-MS analysis

	Name of compound	Relative abundance (%)								
		Control	6-3-3-6	6-1-1-2	6-3-2-5	6-9-1	6-2-5-2	6-1-2	6-9-4	6-2-6-3
1.	Hexadecenoic acid ethyl ester	NA	32,32	33,07	29,37	3,37	3,94	8,26	28,18	23,61
2.	Octadecadienoic acid	40,76	51,58	44,68	40,36	44,27	42,82	49,53	3,27	38,7
3.	Squalene	NA	0,86	0,94	NA	0,59	0,63	NA	NA	0,54
4.	Stigmasta-5,22-dien-3-ol (3 beta) (stigmasterol)	4,05	NA	4	5,15	3,84	3,68	1,07	3,99	3,37
5.	Stigmast-5-en-3-ol (beta-sitosterol)	1,82	NA	NA	0,39	0,59	1,33	NA	2,36	0,39
6.	Eicosane (Icosane)	0,34	0,73	1,62	1,69	NA	1,3	1,45	1,61	2,42
7.	Octacosane	NA	0,58	0,69	NA	2,23	NA	NA	NA	NA
8.	7-pentadecyne	NA	NA	NA	NA	NA	NA	NA	0,48	NA

Note: Chemicals were identified by comparing the retention time with authentic standard database of NIST/EPA/NIH (fit factor ≥90%). NA means it is not available, which means that the quantity of a compound is negligible and cannot be detected by GC-MS. The quantities of anticancer bioactive compounds in MV6 mutant clones which are higher than control are indicated by the yellow highlights

adenocarcinoma (Yoshikawa et al., 2009). Mutant clones' tubers contain greater amounts of at least 5 different anticancer compounds compared with control. The highest increase from control was observed in the chemical profile of clone 6-3-3-6 which contained octadecadienoic acid, 10.82% higher compared with control plant. This compound could induce apoptosis of colon cancer cells (Yoo et al., 2007).

Tubers of mutant clones contain four new anticancer compounds, namely hexadecanoic acid ethyl ester, squalene, octacosane, 7-pentadecyne, which were not found in control plant. Hexadecanoic acid ethyl ester has antioxidant and antimicrobial properties (Bodoprost & Rosemeyer, 2007). In addition, it could reduce the risk of coronary heart disease and cancer (Lai et al., 2008; Bodoprost & Rosemeyer, 2007). Squalene has been proven to be able to inhibit the carcinogenesis of various cancer cell lines, such as colon cancer (Rao et al., 1998). Octacosane was cytotoxic against B16F10-Nex2 skin cancer cells based on *in vitro* experiment (Figueiredo, 2014). The chemical structure of 7-pentadecyne was similar to protein kinase C activator, so it has the potential to be developed into an effective anticancer drugs (Block, 2012). The anticancer activity of 7-pentadecyne has also been confirmed by (Kozikowski et al., 2001).

Tables 6 and 7 also show that mutant clones contain greater amounts of some other chemicals compared with control

plants. Stigmast-5-en-3-ol (3.beta,24s) (beta-sitosterol) is a phytosterol with various biological activities, such as reducing cholesterol level in cells, modify membrane lipid profile (Awad, 1996), anti-diabetic (Sujtha et al., 2010), and inhibit cancer cells (Fraille et al., 2012; Von et al., 1998). Ergost-5-en-3-ol (3 beta) (campesterol) is a phytosterol that could prevent carcinogenesis in lung (Mendilaharsu et al., 1998), gastric (De et al., 2000) and ovarium (Mccann et al., 2003).

Stigmasta-5,22-dien-3-ol (3 beta) (stigmasterol) is antiproliferative against PC3 prostate cancer cells by inducing its apoptosis (Traber & Atkinson, 2007). Stigmasterol could reduce the number of Ehrich Ascites Carcinome (EAC) and is an antioxidant because it could reduce lipid peroxidation and increase glutathione, superoxide dismutase, and catalase in the liver of EAC mice (Ghosh et al., 2011). Another anticancer bioactive compounds of rodent tuber are phenolic compounds (Mohan et al., 2011), pyridine carboxamide (Surjana et al., 2012), octadecanoic acid (Habib et al., 1987), and geranygeraniol (Fernandes et al., 2013).

Rodent tuber's MV1 has undergone protein expression changes compared with control based on 1D and 2D SDS-PAGE analysis (Sianipar et al., 2016). The greater amount of anticancer compounds in mutant clones compared with control has also been observed in MV1 (Sianipar et al., 2016). Leaves and tubers of MV1 contain greater amounts of 3 and 4 anticancer compounds compared control, respectively. Leaves and tubers of MV1 each contains four new anticancer compounds.

According to (Yaycili & Alikamanoglu, 2012), genetic modification of potato plants (alteration of DNA sequence induced by irradiation) can be different between one somatic cell to another. This also happened to rodent tuber MV6 clones. Although they originated from one mother plant, they came from different somatic cells with different genetic make-up. Genetic variation between mutant clones might be due to the difference in DNA repair mechanism or random mutation induced by gamma irradiation (Pillay & Tenkouano, 2011). Genetic variations between each of the mutant clones can result in differences in the chemical contents.

Table 1 shows five mutant clones which had a higher dry weight than control, i.e. clone 6-1-2, 6-9-4, 6-2-6-3, 6-2-4-1, and 6-2-5-2. Four of them, i.e, clone 6-1-2, 6-9-4, 6-2-6-3, dan 6-2-5-2, contained higher amounts of anticancer compounds in their leaves and tubers compared with control (Tables 6 and 7). Those four clones have a potential to be developed into new superior varieties because they have a fast propagation rate and contain a higher amount of valuable anticancer compounds.

CONCLUSION

The chemical compounds in the leaves and tubers of gamma ray-irradiated rodent tubers were higher than the non-irradiated ones. A total of 11 anticancer compounds

were detected in the 6th generation of stable superior mutant clones (MV6) of rodent tuber. Of these, six new anticancer compounds were detected in the leaves and four were detected in tubers. Among the mutant clones, the mutant clone 6-1-1-2 produced the highest amount of new anticancer bioactive compounds. This is the first study of this nature to identify anticancer compound of the 6th generation stable superior mutant clones of rodent tuber using GC-MS method. Therefore, this study should be continued to develop purified bioactive compounds as anticancer drugs.

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