

## Production of Panduratin A, Cardamomin and Sitosterol Using Cell Cultures of Fingerroot (*Boesenbergia pandurata* (Roxb.) Schlechter))

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Fingerroot (*Boesenbergia pandurata* (Roxb.) Schlechter) is an important Southeast Asia especially Indonesian's plant that has many pharmacological activities. This plant contains several secondary metabolites such as flavonoids and their prenylated derivatives like panduratin A that can be produced through tissue culture techniques. This study aimed to produce secondary metabolites mainly flavonoids in the fingerroot cell cultures and explain their biosynthetic relationship. Bud's meristem of fingerroot were initiated to forming callus and shoot in appropriate medium and also induced to produce rhizomes in *in vitro* condition. Panduratin A content was determined using HPLC. The dominant secondary metabolites were isolated and characterized using, chemical reagents and spectroscopic methods. Panduratin A can be produced through callus and rhizomes formation of fingerroot. Other two secondary metabolites have been identified as phytosterol and cardamonin with the content  $285.85 \pm 8.36\%$  and  $316.35 \pm 0.82\%$  respectively which were higher than the original plant. Panduratin A, phytosterol and cardamonin which are supposed to have pharmacological activities have been produced using cell culture of fingerroot *Boesenbergia pandurata* (Roxb.) Schlechter with higher level compared to the original plants.

**Key words:** *Boesenbergia pandurata* (Roxb.) Schlechter, Panduratin A, Cardamomin, fingerroot, phytosterol, tissue culture.

Secondary metabolites are chemical compounds that were produced through synthesis, degradation, and rearrangement process of primary metabolite that was typical and occur in particular organisms. Secondary metabolites have many functions such as defense compounds, odor and provide color in plants. Secondary metabolites also have pharmacological activity, so that can be used as a drug (Dewick, 2009). A potential medicinal plant which produces the secondary metabolites is fingerroot (*Boesenbergia pandurata* (Roxb.) Schlechter). Fingerroot is one of the typical plants

of Southeast Asia especially Indonesia, which has long been used as a spice and medicinal raw materials. Fingerroot reported has activities as antipyretic, analgetic, antiinflammatory (Tewtrakul *et al.*, 2009), anti human immunodeficiency virus-1 (HIV-1) protease (Cheenpracha *et al.* 2006), antibacterial (Rukayadi *et al.*, 2009; Rukayadi *et al.*, 2010; Yanti *et al.* 2009), and potential as cytotoxic and chemotherapeutic agent (Sarmoko *et al.*, 2008; Sukari *et al.*, 2007; Cheah *et al.* 2001). Flavonoids, especially chalcone groups such as pinostrobin, pinocembrin, alpinetin, cardamonin, boesenbergin A, 2'-hydroxy-4,4',6'-trimethoxy chalcone, 5-hydroxy-7-methoxy flavanon, 5,7-dihydroxyflavanon, 2',6',-dihydroxy-4'-methoxy chalcone, 4-hydroxypanduratin A, panduratin A, and panduratin C (Tewtrakul *et al.*, 2009; Shindo *et al.*, 2006), and also Panduratin

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D-I (Win *et al.*, 2008), have been reported to play the important role in the pharmacological activities. Biotechnological approaches (*in vitro*), especially plant tissue culture have been used as an alternative to produce useful secondary metabolites as medicines from plants and give several advantages (Karuppusamy, 2009). One of the advantages of this method is the production cycle period is very short and repeated between 2 to 4 weeks. In addition, *in vitro* production of secondary compounds can be carried anywhere without depending on climatic factors. Under *in vitro* conditions, the plants can produce secondary metabolites with different compositions than wild type plants. Even in *in vitro* conditions, the plants can produce new compounds that were not found in wild type before. This study aimed to produce valuable secondary metabolites from fingerroot under *in vitro* conditions compared with wild type and explain their biosynthetic connection (Karuppusamy, 2009).

## MATERIALS AND METHODS

### Sample preparations

Healthy fingerroot's rhizomes were collected from Kebun Percobaan Manoko, Lembang, Bandung, placed on open basket until bud sprouting to 1-2 cm in length. Buds as explants were cut and cleaned under water flow, soaked in soap solution for an hour, and rinsed. Further, the explants were soaked in ethanol 70 % v/v for 1 min, followed soaking in NaOCl 3 % v/v (bayclin) and 2 drops tween 80 for 20 min with agitation. In laminar air flow cabinet (Holten®), explants were rinsed in steril destilated water 5 times, 5 min respectively. Bud's meristems were cut aseptically into 4 pieces laterally and then placed into 9 cm Petri dish containing 25 mL of Murashige and Skoog (MS) (Phytotechlab®) basal medium.

### Culture media and conditions

Murashige & Skoog media was supplemented with other appropriate components based on test condition. Solid media was made by addition agar 0,7 % b/v. Before sterilized in autoclave at 121 °C, 1 atm pressure, for 20 min, medium's pH was adjusted with HCl 1N or NaOH 1N to reach 5,8. Media was cooled before used. All culture were incubated at temperature 20 ± 2 °C and photoperiode 8/16 (dark/light). Inoculum

in liquid medium was incubated on orbital shaker at 100 rotation per min.

### Inoculum initiation

Healthy explant was initiated to produce callus using solid MS medium supplemented with 30g/l sucrose and growth regulators (GR) such as 2,4-dichlorophenoxyacetic acid (2,4 D),  $\alpha$ -naphthaleneacetic acid (NAA), indole butyric acid (IBA), benzylaminopurine (BAP), and kinetin (KIN) at level 3 mg/l. Cultures were collected after 6 weeks incubation. Friable callus were subcultured into liquid MS medium supplemented with 0,5 mg/l NAA as growth regulator, 3 fold MS vitamin and 30 g/l sucrose. Callus were induced to shoots forming, then subcultured onto solid MS medium supplemented with 0,5 mg/l NAA and 30g/l sucrose.

### Rhizomes formation

Eight weeks ages healthy shoots were induced to rhizomes formation by subcultured into 4 different solid MS medium. First media supplemented with 1 mg/l BAP + 0,1 mg/l IBA, second media supplemented with 1 mg/l BAP + 0,2 mg/l IBA, third media supplemented with 1 mg/l BAP + 0,1 mg/l NAA, and fourth media supplemented with 1 mg/l BAP + 0,2 mg/l NAA. All medium containing 90g/l sucrose as carbon source. Rhizomes induction also were done in 4 liquid medium that was supplemented with growth regulators as above.

### Phytochemical analysis

#### Preparation of extracts

Phytochemical study were done by qualitative analysis at first. A number of 50 mg of dried sample (callus, root, rhizome, leaf *in vitro*, and wild type rhizome) was macerated in 5 mL methanol for 3 times 24 hours respectively. Methanol extracts were combined and evaporated. Residue were redissolved in 500 $\mu$ L methanol (Merck®).

Each extracts were spotted on a thin layer chromatography (TLC) plate (silica G 60 F254 precoated, Merck®) at the same volume and developed with n-hexane: ethyl acetate (8:3) as the mobile phase. After air drying, TLC plate was analyzed under UV lamp at 254 nm and 366 nm. Metabolites on the plate were also detected by spraying H<sub>2</sub>SO<sub>4</sub> 10% v/v and heated. Further, the more dominant spots that were present at *in vitro* samples than wild type, were isolated and characterized.

### Isolation and characterization

Milled callus (10 g) and *in vitro* rhizome (5,5 g) were macerated in chloroform and methanol respectively for three times with agitation at room temperature. The extracts were evaporated to get semisolid extract. Each extract then fractionated by preparative TLC (1 mm, silica G 60 F254, Merck®) with n-hexane: ethyl acetate (8:3) as the mobile phase. The selected band for each plates were scraped and extracted with ethyl acetate continued with methanol for three times respectively. After recrystallization with methanol and n-hexane, callus extract's fraction provided a needles white crystal (isolate 1, 1.2 mg), while *in vitro* rhizome extract's fraction provided a yellowish prism crystal (isolate 2, 5.6 mg). The isolates were then characterized by TLC with reference compounds, spraying reagents, and spectroscopy such as spectrophotometry UV-Vis (Beckman®), <sup>1</sup>H-NMR and <sup>13</sup>C-NMR (Agilent® series 500 MHz NMR).

### Quantitative analysis of Panduratin A and comparison content of isolates 1 and 2

Panduratin A content was determined by HPLC apparatus (Hewlett Packard® series 1100) using ODS Hypersill 5µm column (100 mm × 4,6 mm, Hewlett Packard®) at 30°C and monitored at 280 nm and 340 nm. Two eluents, namely 0,5% v/v formic acid (eluent A) and acetonitrile (eluent B) was used as mobile phase. Gradient elution was carried out initiated by 20 % of eluent B, linear gradient to reach 40 % of eluent B at 5 min., linear gradient to reach 70 % of eluent B at 15 min, linear gradient to reach 80 % of eluent B at 20 min. Followed to reach 85 % of eluent B at 21 min. The column was returned to initial condition at 22 min. The flow rate was 0.6 mL/min and 20 µL samples and reference (panduratin A) were injected after through 0.22 µm membrane filter. Quantification of panduratin A in samples was calculated from the calibration curve of panduratin A.

Comparison content of isolate 1 and 2 was performed by spectrophotodensitometry. Samples were prepared as describe above. All samples include isolate 1 and 2 were spotted on TLC plate using TLC sample applicator (Camag® linomat 5) about 3 µL. After developed with n-hexane: ethyl acetate (8:3), TLC plate was then scanned in spektrofotodensitometer (Camag® TLC scanner 3) at 340 nm to analyze isolate 2, and 455 nm to analyze isolat 1 after derivatisation with H<sub>2</sub>SO<sub>4</sub> 10

% v/v and heated for 2 minutes. Data as area under curve (AUC) of each peak with corresponding R<sub>f</sub> values and spectra to the isolates were determined and then compared.

## RESULTS

### Callus initiation and rhizomes induction

Application of different growth hormone giving a different effect to the explant as shown on **Table 1** and **Fig. 1**. Induction with 3 mg/L<sub>2,4</sub> D produced yellowish and friable callus as much as 20.00 % from induced explants. Use of 3 mg/L IBA also produced callus at same mounts, but not friable and showed brown colour. Different with the other auxin, NAA at levels 3 mg/L did not form callus. This hormone induced shoots only as much as 86.67 %. Cytokine such as kinetin (KIN) and BAP were only produce shoots about 6.67 and 40.00 % respectively. The friable callus as inoculum then was subcultured to liquid medium that was optimized before (Fig. 2. A). In liquid medium, callus showed better growth index than on solid medium.

Eight weeks old shoots then were moved to eight different medium to induce rhizomes (Fig. 2. B). All medium contain 90 g/L sucrose as carbon source. This condition was selected based on optimal sucrose level that was used to induce rhizomes in *Curcuma longa* L. (Zingiberaceae) (Islam *et al.*, 2004). Results indicated that the combination of growth hormone had an effect on *in vitro* rhizomes induction (Table 2). Among 8 different medium treatments, liquid MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l IBA provided the best response than the other medium treatment in term the mean dry weight ( $157.8 \pm 31.12$  mg) followed by 1.0 mg/l BAP and 0.2 mg/l NAA as the second ( $149.0 \pm 21.38$  mg). On solid medium, combination of growth hormones 1.0 mg/l BAP and 0.1 mg/l IBA gave the highest dry weight ( $58.2 \pm 6.04$  mg) followed by 1.0 mg/l BAP and 0.2 mg/l NAA as the second ( $53.7 \pm 7.60$  mg). Revealed, that the use of combination IBA with BAP, both in liquid and solid media were able to induce the rhizome more optimal than NAA and BAP combination (Fig. 2. C).

### Phytochemical analysis

Analysis of each extract on the TLC plate exhibit a different chromatogram pattern (Fig. 3).

**Table 1.** Influence of several plant growth regulator on fingerroot explants

PGR (3.0 mg/l)	n	Callus (%)	Shoot (%)	Root (%)	Keterangan
2.4 D	25	20.00	76.00	0.00	Yellowish calli, Friable
IBA	15	20.00	0.00	13.33	Brown calli, Compact
NAA	15	0.00	86.67	0.00	Each explant produce 1-2 shoots with roots
KIN	15	0.00	6.67	0.00	Small shoots with root
BAP	15	0.00	40.00	0.00	Each explant produce 1 shoots with roots

**Table 2.** Influence the growth regulator combination on fingerroot's rhizomes dry weight

Medium consistency	Dry weight of rhizomes (mg)			
	1.0 mg/l BAP			
	0.1 mg/l IBA	0.2 mg/l IBA	0.1 mg/l NAA	0.2 mg/l NAA
Liquid	157.8±31.12	147.9±37.68	147.6±23.51	149.0±21.38
Solid	49.7±12.54	58.2±6.04	48.0±4.99	53.7±7.60

**Table 3.** Chemical characterization results of isolat 2

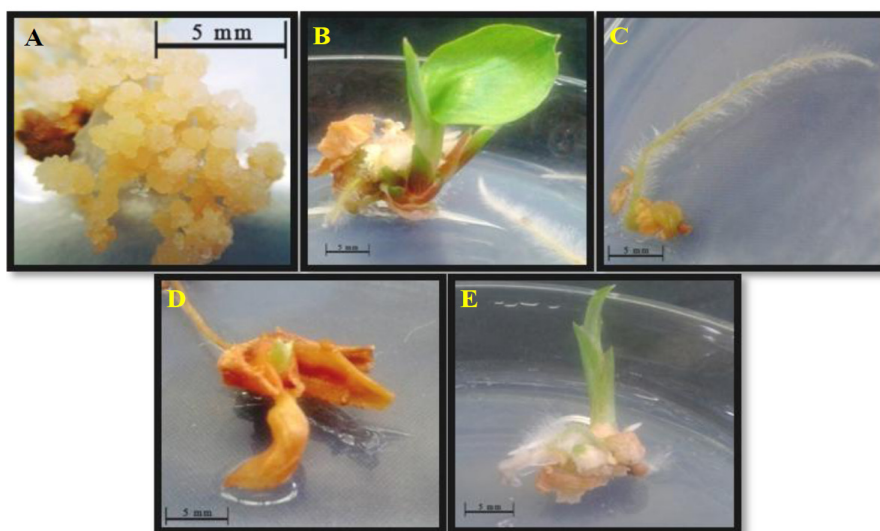
Chemical reagent	Detection under	Results
Initial	Visible light/UV <sub>366 nm</sub>	Yellow/Extinction
NH <sub>3</sub> vapour	Visible light/UV <sub>366 nm</sub>	Increase yellow intensity/Increase extinction
Citroboric	UV <sub>366 nm</sub>	Increase extinction
5% FeCl <sub>3</sub>	Visible light	Reddish colour
5% AlCl <sub>3</sub>	Visible light	Increase yellow intensity
Anisaldehyde	Visible light	No change

**Table 4.** Panduratin A content in fingerroot's sample

Samples	Panduratin A contents (µg/g)
<i>wild type</i> rhizomes	211.70±6.08
Callus	6.86±0.03
<i>in vitro</i> rhizomes in solid medium	105.44±1.30
<i>in vitro</i> rhizomes in liquid medium	148.32±9.15

**Table 5.** Comparison of isolates content in samples to wild type rhizomes of fingerroot

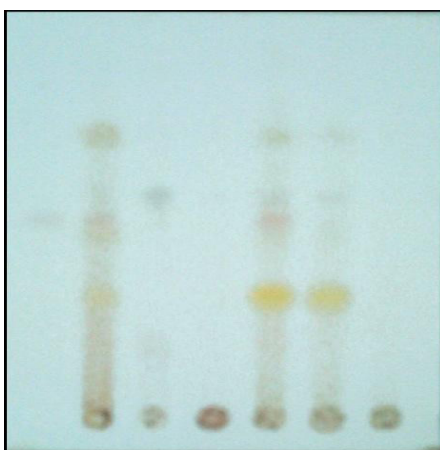
Samples	Phytosterol (%)	Cardamonin (%)
<i>in vitro</i> roots	86.00 ± 17.88	nd
<i>in vitro</i> rhizomes	150.31 ± 6.55	316.35 ± 0.82
<i>in vitro</i> leaves	nd	nd
Callus	285.85 ± 8.36	nd
Wild type rhizomes	100	100



**Fig. 1.** Influence of several plant growth regulator at level 3.00 mg/l on fingerroot explants, (A) 2,4 D, (B) NAA, (C) IBA, (D) Kinetin dan (E) BAP.



**Fig. 2.** (A) Friable callus of fingerroot in liquid medium, (B) Macroscopic of fingerroot plantlet, in (left) solid medium and (right) liquid medium, (C) Rhizome that was induced from shoot *in vitro*



**Fig. 3.** TLC Chromatogram under visible light after 10%  $H_2SO_4$  spraying and heated; (1) panduratin A, (2) wild type rhizome, (3) Callus, (4) *in vitro* root, (5) *in vitro* rhizomes in liquid medium, (6) *in vitro* rhizomes in liquid medium, (7) *in vitro* leaf; developed with n-hexane : ethyl acetate (8:3)

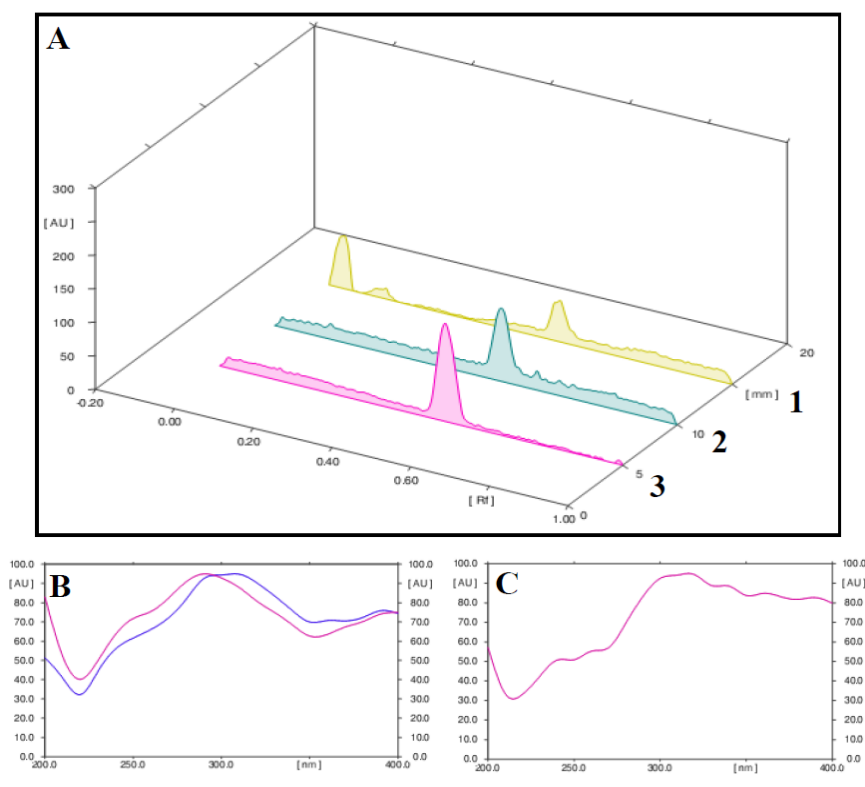
On *in vitro* rhizome, both liquid and solid induction, showed a bigger yellow spot than on wild type at Rf 0.33. While, on callus extract showed a bigger reddish grey spot than wild type at Rf 0.55. The *in vitro* root and leaf extract did not show any spots on the development system. Furthermore, these two metabolites will be isolated and characterized.

#### Characterization of isolates

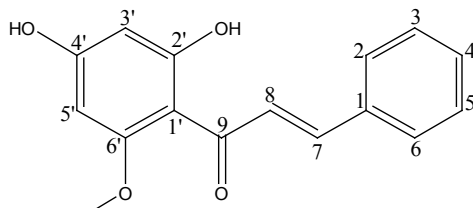
The first isolate (1) was obtained from callus extract, appear as needles white crystal (1.2 mg). Characterization of isolate 1 by spectrophotometer UV-Vis, shown an absorbance at

$\lambda_{\text{maks}}$  (MeOH) 204 nm. This data suggest that isolate 1 did not has a chromophore. Monitoring of isolate 1 by TLC with steroidal reference compounds such as stigmasterol and sitosterol, shown that isolate 1 had an identic Rf value and spot pattern (Fig. 4. A) with them. Substantial assumption that isolate 1 was steroid compounds. Spectral scanning's results by spectrophotodensitometer, showed that isolate 1 had identical spectra with sitosterol (Fig. 4. B and C).

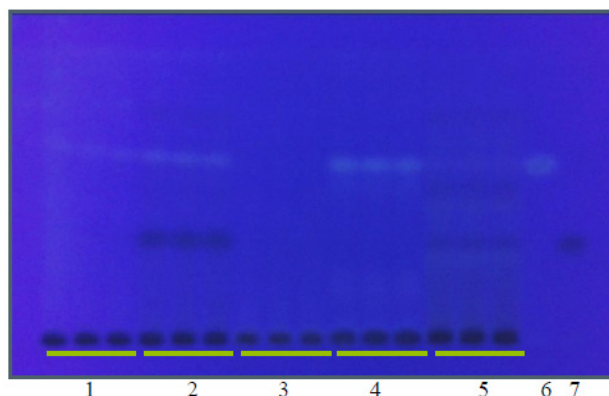
The second isolate (2), obtained from *in vitro* rhizomes extract, provided as a yellowish



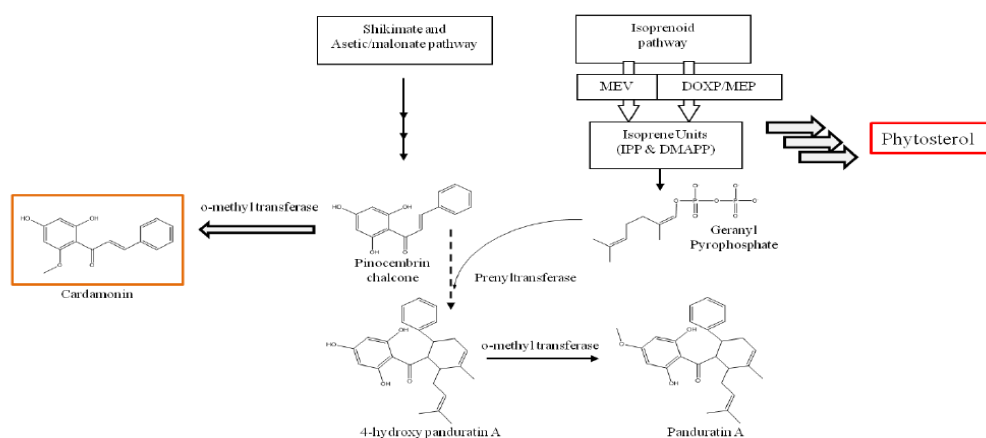
**Fig. 4.** (A) TLC Chromatogram with reference compounds after derivatization by 10 %  $\text{H}_2\text{SO}_4$  and heated, (1) isolate 1, (2) sitosterol, (3) stigmasterol; (B) UV spectra of reference compound, ( ) sitosterol and ( ) stigmasterol, (C) UV spectra of isolate 1



**Fig. 5.** Chemical structure of 2',4'-dihydroxy-6'-methoxy chalcone (cardamonin)



**Fig. 6.** TLC Chromatogram under UV<sub>366 nm</sub> light after derivatization by 10 % H<sub>2</sub>SO<sub>4</sub> and heated, (1) *in vitro* root, (2) *in vitro* rhizomes, (3) *in vitro* leaf, (4) callus, (5) wild type rhizomes, (6) isolate 1, (7) isolate 2; developed with n-hexane : ethyl acetate (8:3)



**Fig. 7.** Putative relationship biosynthetic pathway of cardamonin, phytosterol and panduratin A

prism crystal (5.6 mg). Chemical characterization of isolate 2 by various spraying reagent, revealed that isolate 2 gave positive result with NH<sub>3</sub> vapour, citroboric, 5% FeCl<sub>3</sub>, and 5% AlCl<sub>3</sub>, while negative result was only shown by anisaldehyde reagent as describe in Table 3. Those data revealed that isolate 2 was a phenolic compound, and probably it was a flavonoid. Characterization by spectrophotometer UV-Vis, isolate 2 showed an absorbance at  $\lambda_{\text{maks}}$  (MeOH) 205nm, 342 nm and a shoulder at 234 nm. This data suggested that isolate 2 has a chromophore. The spectrum of isolate 2 at UV range, followed the flavonoid pattern especially chalcone spectra.

<sup>13</sup>C-NMR (125 MHz, aseton-d<sub>6</sub>) data showed a carbonyl carbon signal at  $\delta_{\text{C}}$  193.20 ppm and three oxaryl carbon signal at  $\delta_{\text{C}}$  169.05, 166.06,

and 164.44 ppm and also a methoxyl carbon signal at  $\delta_{\text{C}}$  56.49 ppm. Beside those signal, there was also appeared 11 carbon signal at aromatic range.

<sup>1</sup>H-NMR (500 MHz, aseton-d<sub>6</sub>) data revealed a methoxyl signal at  $\delta_{\text{H}}$  3.99 (3H, s) ppm. Two proton signal with meta correlation were also appeared at  $\delta_{\text{H}}$  6.01 (1H, d,  $J=2.2\text{Hz}$ ) and  $\delta_{\text{H}}$  6.10 (1H, d,  $J=2.4\text{Hz}$ ) ppm. In addition, the aromatic region was also contained three signals at  $\delta_{\text{H}}$  7.40 – 7.48 (3H, m),  $\delta_{\text{H}}$  7.71 – 7.77 (3H, m) and at  $\delta_{\text{H}}$  8.01 (1H, d,  $J=15.6\text{ Hz}$ ) ppm. With the data 1D <sup>1</sup>H-<sup>1</sup>H TOCSY, it was found that the signal at  $\delta_{\text{H}}$  8.01 (1H, d,  $J=15.6\text{ Hz}$ ) ppm correlated with the signal at 7.75 (1H, d,  $J=15.5\text{ Hz}$ ) which was overlap as a multiplet signal at  $\delta_{\text{H}}$  7.71 - 7.77 ppm. Coupling constant's value showedn that those signal were correlated as *trans* olefinic position. Five proton at

aromatic region that appeared as multiplets signal at  $\delta_H$  7.71 – 7.77 and  $\delta_H$  7.40 – 7.48 ppm suggested from monosubstituted phenyl group.

Based from those data, it can be concluded that isolate 2 was a clachone with one methoxyl substitution. Conformity  $^{13}\text{C}$ -NMR and  $^1\text{H}$ -NMR datas of isolate 2 with isolate datas obtained by literature showed that isolate 2 was 2', 4'-dihydroxy-6'-methoxy chalcone (cardamonin) (Fig. 5) (Jaipetch *et al.*, 1982; Ching *et al.*, 2007). Cardamonin was not a new compound. This compound has been isolated for first time from *Alpinia katsumadai hayata*'s seed and also been reported present in fingerroot's rhizomes (Jaipetch *et al.*, 1982; Ching *et al.*, 2007; Kimura *et al.* 1968).

#### **Quantitative analysis of panduratin A and comparison content of isolates 1 and 2**

Panduratin A was determined using HPLC as describe on material and methode. Table 4 showed the different panduratin A contents in each sample that was injected. Data showed wild type rhizomes contains the highest panduratin A among the other, while callus sample was the smallest. Induction *in vitro* rhizomes in liquid medium resulted a higher panduratin A contents than on solid medium.

Comparison isolate 1 and 2 content in *in vitro* sample to wild type was shown in Table 5. For sitosterol, the highest content was found in callus about  $285.85 \pm 8.36$  % higher than wild type. Sitosterol was also detected in *in vitro* rhizomes and roots about  $150.31 \pm 6.55$  % and  $86.00 \pm 17.88$  % respectively, higher than in wild type. Cardamonin as describe in Fig. 6, was only detected in rhizomes sample, either *in vitro* nor wild type. Highest content was found in *in vitro* sample about  $316.35 \pm 0.82$  %.

### **DISCUSSION**

The efforts of fingerroot plants propagation through tissue culture techniques have been reported, either through direct or mediated through the callus formation (Yusuf *et al.* 2011a; Yusuf *et al.*, 2011b). In this present study were found that healthy callus can be induced using growth hormone 3.00 mg/l 2,4 D. Yusuf *et al.* (2011) also reported that callus can be induced by 2,4 D at levels of 2.00 mg/l (Yusuf *et al.*, 2011b). While, Tan *et al.* (2012) had reported callus induction is

done with a combination of several types of auxin such as 2,4 D, NAA and IAA at levels 1 mg/l for each (Tan *et al.*, 2012).

Panduratin A as one kind of marker for this plants, was made up from chalcone cluster and geranyl (two isoprene) as side chain. This compound had been reported has many pharmacological activities such as antiinflammatory, antibacterial, anti mutagenic, even as anti viruses (Tewtrakul *et al.*, 2009; Cheenpracha *et al.* 2006; Rukayadi *et al.*, 2009; Rukayadi *et al.*, 2010; Yanti *et al.* 2009; Sarmoko *et al.*, 2008; Sukari *et al.*, 2007; Cheah *et al.* 2001). So this compound was remarkably potential to produced via tissue culture technique. In this present study, panduratin A can be produced in callus and *in vitro* rhizomes. Although the product that was produced still lower than the wild type. But several effort can be applied to enhance the yield.

Sucrose at high level can be used to induce rhizomes forming in shoots *in vitro*. Many other reports had stated that high level of sucrose, remarkably promotes the *in vitro* forming of storage organ such as bulbs, tubers, and in this case rhizomes (Islam *et al.* 2004). The present of growth hormone in medium remarkably help rhizomes formation. In the present study showed that the use of 1 mg/l BAP combined with IBA, gave a good results.

Based on the phytochemical analysis in the present study showed that at *in vitro* condition, fingerroot had a slight dominant biosynthetic pathway shifting. In callus, the dominant metabolites that was produced is sitosterol (as one of the phytosterol group). Steroid group had so many role in plant cells, such as growth hormones and cell wall component. In *in vitro* condition, especially callus, plant cell was stimulated to growth and propagate rapidly, so the steroid level was enhanced too. The high osmotic condition in media, also promotes plant cells to keep their integrity through the establishment of the cell wall. Steroid group were made from isoprenoid trough mevalonate pathway. The chemical structure of panduratin A were also need isoprenoid group as the side chain. Due to the domination of the mevalonate pathway, give an impact to the decrease of isoprenoid availability in callus, so the level of panduratin A is low. In other case, in *in vitro* rhizomes, the dominat biosynthetic pathway

that was appear is the cardamonin formation. Cardamonin is a chalcone that was methoxylated at 6' position by o-methyl transferase enzymes. As describe above, panduratin A were made up from chalcone and isoprene structure. The chalcone cluster of panduratin A was also had a methoxy group, but in different position, that is at 4' position. The high level of cardamonin in *in vitro* rhizome of fingerroot, provided an opportunity to produce these compounds through tissue culture techniques. Literatures study had been reported that cardamonin also has so many pharmacological activity, such as anticancer (Qina *et al.* 2012; Yadav *et al.*, 2012; Park *et al.* 2013), antiinflammatory effect (Takahashi *et al.*, 2011; Chow *at al.*, 2012), and as vasodilator (Fusi *et al.*, 2010), so this compounds could be developed as pharmaceutical raw material, and produced through plant biotechnology techniques.

Putative biosynthetic pathway of these compound was assumed as described in Fig. 7. Based on allegations the biosynthetic pathway, by regulate the expression of the enzymes that were responsible to the certain pathway, then the production of certain secondary metabolites can be improved. In this case, production of panduratin A can be improved by decrease the expression of squalene synthase and chalcone 6' o-methyl transferase and enhance the expression of chalcone spesific prenyltransferase.

## CONCLUSIONS

Tissue culture was a remarkably techniques that can be used to produce panduratin A, phytosterol and cardamonin Considering their valuable activities, the production through cell cultures can be an alternative sources for the production of the compounds for herbal medicine purposes. Based on their biosynthetic relationship, levels of one compound may be increased by regulate their biosynthetic pathways.

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