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African Journal of Biotechnology

Full Length Research Paper

Antioxidant, cytotoxic, and antidiabetic activities of *Dendropanax morbifera* extract for production of health-oriented food materials

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Antioxidant, cytotoxic and anti-diabetic effects of fermented and non-fermented *Dendropanax morbifera* extracts were compared to assess the potential utility of this species in the development of health-oriented food. The non-fermented extract (NFDE) was obtained from leaves and branches of *D. morbifera* and the fermented extract (FDE) was prepared by inoculation with *Lactobacillus plantarum* and *Lactobacillus brevis* after extraction of *D. morbifera* with distilled water. Antioxidant activity before and after fermentation was assessed via the α, α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay, cytotoxicity analyzed with the MTT assay using 3T3-L1 cells and anti-diabetic activity measured based on inhibition of α-glucosidase activity. The *D. morbifera* extract exhibited substantial antioxidant activity. Moreover, FDE at 24 h exerted more significant antioxidant effects than NFDE (97.1 vs 89.8%) at a concentration of 5 mg/ml. Comparison of the effects of the non-fermented and fermented extracts on 3T3-L1 cell viability revealed slightly higher cytotoxicity of FDE than NFDE (85 vs 95% viability) at a concentration of 500 μg/ml. Both NFDE and FDE (100 μg/ml) exerted strong α-glucosidase inhibitory effects (98.9 and 97.6%, respectively). In view of the low cytotoxicity coupled with significant antioxidant and anti-diabetic effects, the *D. morbifera* extract presents a novel candidate for the production of functional anti-diabetic agents with minimal side-effects.

Key words: *Dendropanax morbifera*, fermented and non-fermented extracts, antioxidant activity, cytotoxicity, antidiabetic effects, health-oriented food.

INTRODUCTION

Due to westernized eating habits and lack of exercise, the incidence of obesity and diabetes continues to rise by >10% every year (Xu et al., 2011). Increasing intake of high-calorie meals has resulted in a growing number of

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patients with metabolic syndrome diseases, such as diabetes and hyperlipidemia. In 2014, diabetes was the sixth most common cause of death in Korea. Diabetes, a type of metabolic disease characterized by hyperglycemia with elevated blood glucose levels, is caused by lack of insulin secretion in pancreatic cells or failure of normal kidney function (Li et al., 2013). In particular, oxidative stress is associated with progression of diabetes and contributes significantly to complications (Brownlee, 2005). Under conditions of long-term persistence of hyperglycemia, reactive oxygen species (ROS) produced during glycosylation of glucose enhance lipid peroxidation and oxidative damage, leading to various diabetic complications, such as hypertension, arteriosclerosis and hyperlipidemia (Sakurai and Tsuchiya, 2006; Lones, 1991; Tai et al., 2000).

Continuous efforts to improve metabolic syndromes through ingestion of specific dietary components are underway. Current diabetic includes sulfonylurea, metformin, alpha-glucosidase inhibitor, thiazolidinedione and dipeptidyl peptidase-4-inhibitor as well as insulin. The chemical drugs currently available for treatment of diabetes cause serious side-effects, highlighting the necessity to develop effective natural remedies.

Recently, *Dendropanax morbifera* has been increasingly cultivated on Jeju Island and some regions of the Korean coastline along the southwestern sea. *D. morbifera*, a subtropical broad-leaved evergreen tree belonging to the family Araliaceae, is an economically important species due to its use in the production of golden varnish (Moon et al., 1999; Kim et al., 2006). In addition, its leaves, stems, roots and seeds are traditionally used in folk medicine for skin and infectious diseases, headaches and other maladies (Park et al., 2004). Various beneficial physiological activities of *D. morbifera* have been documented, such as improvement of lipid abnormalities, diabetic disease, immune activity, thrombosis and kidness losss protection effect (Tan and Ryu, 2015; An et al., 2014; Lee et al., 2002; Choi et al., 2015; Kim et al., 2015). The plant is additionally reported to exert a skin whitening effect (Park et al., 2014; Lee et al., 2015), indicative of a variety of physiologically active components. However, limited information is available on the potential anti-diabetic effects of *D. morbifera*.

Most of the foods using *D. morbifera* are beverages, which are produced by simple processing or by hot water extraction or natural fermentation using sugar. However, in this study, it is intended to develop a health-oriented food materials which can differentiate from the similar products through fermentation of lactic acid bacteria as a raw material and produce antioxidant and antidiabetic activities of *D. morbifera* extract. This study focused on evaluation of the antioxidant, cell toxicity and anti-diabetic activities, in particular, alpha-glucosidase activity of *D. morbifera* distilled water extracts. Our results may serve as a platform to evaluate the utility of *D. morbifera* extracts as a nutraceutical source for management of diabetes in the future.

MATERIALS AND METHODS

Preparation of *D. morbifera* extracts

Boughs of *D. morbifera* were collected from a natural habitat in Jeju Island in February 2016. Samples were dried at room temperature and subjected to the extraction process. The collected *D. morbifera* boughs were cut into 1.0 cm length sections. The distilled water extract of *D. morbifera* (NFDE) was extracted with 20 volumes of water at 95°C for 4 h and reduced to a powder using the spray-dry method. Fermented *D. morbifera* (FDE) was prepared as follows: *L. plantarum* and *L. brevis* were inoculated in De Man, Rogosa and Sharpe (MRS) broth at 37°C for 24 h and diluted to obtain an initial population of 1-5 × 10^7 CFU/ml. *D. morbifera* solution (5%) was inoculated with fresh bacterial subculture (4% v/v) for fermentation at 37°C for 24 h, followed by sterilization and filtration. The filtered solution of fermented sample was concentrated using a rotary evaporator and spray-dried.

Total phenolic assay

The total phenolic content was determined with the Folin-Ciocalteu assay (Singleton and Lamuela-Raventos, 1999) using gallic acid (GA) as the standard. A mixture comprising of the sample solution (50 µl), distilled water (3 ml), 250 µl Folin-Ciocalteu's reagent solution, and 7% $NaCO_3$ (750 µl) was vortexed and incubated for 8 min at room temperature, followed by dilution into 950 µl distilled water. The mixture was allowed to stand for 2 h at room temperature and absorbance measured at 765 nm against distilled water as a blank. Total phenolic content was expressed as gallic acid equivalents (µg GAE/ml sample) based on a gallic acid calibration curve. The linear range of the calibration curve was 10 to 200 µg/ml (r = 0.99).

Measurement of antioxidant activity of extracts

The antioxidant capacity of extracts was analyzed by measuring free radical scavenging activity using the DPPH assay (Brand-Williams et al., 1995). Samples were prepared at concentrations of 0.1, 1 and 5 mg/ml. Vitamin C treatment was used as the positive control group. After maintaining at room temperature for over 30 min, free radical scavenging activity was determined by mixing with 500 µM DPPH solution (1:1) and incubating in the dark, followed by measurement of absorbance at 517 nm using a spectrophotometer.

Analysis of cytotoxicity of extracts

3T3-L1 mouse preadipocytes obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea) were used for cytotoxicity experiments. Preadipocyte cells were sub-cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (P/S; Gibco) every 24–36 h and seeded in 96- well plates at a density of 1.0 × 10^4 cells per well. Cells were treated with 200 µl NFDE and FDE at a range of concentrations (0, 50, 100, 200, 300, and 400 µg/ml) and incubated at 37°C for 4 h in 5% CO_2. Cell viability was determined according to the protocol provided by the manufacturer. MTT reagent (20 µl) was added to individual wells and incubated under similar conditions for 1 h. Absorbance of plates was read at 490 nm in a microplate reader. The number of viable cells was directly proportional to absorbance

Table 1. Total polyphenol contents in distilled water and fermented extracts of *D. morbifera*

Variable	NFDE	FDE			
	0 h	12 h	24 h	48 h	72 h
Polyphenol (µg GAE/ml)	562.44±13.2	605.75±14.2	625.30±14.7	630.21±14.8	640.03±15

Fermentation time at 37°C, GAE: gallic acid equivalent. Values represent means ± SD (n=3).

of formazan formed due to reduction of MTT. Cell viability was expressed as a percentage of control cells. All experiments were performed in triplicate.

Analysis of alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity of the extract was examined according to a standard protocol with minor modifications (Shai et al., 2011). The reaction mixture containing 50 µl phosphate buffer (100 mM, pH 6.8), 10 µl alpha-glucosidase (1 U/ml) and 20 µl of various concentrations of extract (0, 10, 20, 50 and 100 µg/ml) was preincubated in a 96-well plate at 37°C for 15 min. Next, 20 µl *p*-nitrophenol (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was terminated with the addition of 50 µl Na_2CO_3 (0.1 M). Absorbance of released *p*-nitrophenol was measured at 405 nm using a multiplate reader. Acarbose (0.1~0.5 mg/ml) was included as a standard. A control sample without the test substance was set up in parallel, and each experiment performed in triplicate. Results were expressed as percentage inhibition calculated using the formula:

Inhibitory activity (%) = (1 − As/Ac) ×100

where, As represents absorbance of the test sample and Ac the absorbance of control.

Statistical analysis

All data are presented as mean ± STD. Differences among treatments were assessed by analysis of variance (ANOVA), followed by Dunnett's test. p values V 0.05 were considered to be significant.

RESULTS

Total polyphenol content

Two type strains, *L. plantarum* and *L. brevis* were investigated as starter cultures for the fermentation of *D. morbifera*. Following fermentation, total phenolic contents and antioxidant activities of fermented *D. morbifera* using starter cultures were determined. The total phenolic contents of NFDE and FDE were measured using a standard curve prepared with different concentrations of gallic acid. In the current study, the total phenol contents of NFDE and FDE were determined as 562.44 and 640.03 µg/ml, respectively, and shown to increase with fermentation time (Table 1). It was confirmed that the total polyphenol contents had expanded by about 1.14 times in the case of FDE compared to NFDE. Earlier, Kang et al (2011) reported that the phenol content is increased by fermentation at 8.13 and 9.53 mg/ml in extract of *Maclura tricuspidata* and the fermented extract of *M.tricuspidata*, respectively.

DPPH radical scavenging activity of *D. morbifera* extracts

Comparison of DPPH radical scavenging abilities before and after fermentation according to extract concentration showed higher inhibitory activity of FDE than NFDE (Table 2). NFDE exerted increasing inhibitory effects (10.68, 65.31, and 89.8%) at concentrations of 0.1, 1, and 5 mg/ml, respectively. Within this concentration range, the inhibitory effects of FDE at 24 h were 13.67, 72.61, and 97.1%, respectively. It was confirmed that DPPH radical scavenging had increased around 1.08~1.28 times in the case of FDE compared to NFDE.

Effects of *D. morbifera* extracts on 3T3-L1 cell viability

This study aimed to discover a possibility that NFDE and FDE can be used as health-oriented food materials. To determine the effects of the extracts on cell viability, the MTT assay was performed on 3T3-L1 cells treated with 0 to 500 µg/ml NFDE or FDE. The results are expressed as a percentage of surviving test cells relative to the control group (Figure 1). No significant toxicity in 3T3-L1-differentiated cells treated with both fermented and non-fermented extracts at a range of concentrations was observed (0 to 500 µg/ml).

α-Amylase inhibitory activity

This study examined the inhibitory activity of *D. morbifera* extract against α-amylase to evaluate the availability of *D. morbifera* extract as a functional food agent. In the α-amylase assay, the inhibitory effects of NFDE and FDE on α-glucosidase activity increased in a concentration-dependent manner (Figure 2). The inhibitory effects of NFDE and FDE on α-glucosidase increased in a concentration-dependent manner (43, 53.5 97.5 and 98.9% at NFDE concentrations of 10, 20, 50, and 100 µg/ml and 42.1, 52.1, 96.5, and 97.6% at FDE concentrations of 10, 20, 50, and 100 µg/ml, respectively).

Table 2. DPPH radical scavenging activity (%) of distilled water and fermented extracts of *D. morbifera*

Sample	Concentration (mg/ml)		
	0.1	1	5
NFDE	10.68±2	65.31±2.5	89.8±0.2
FDE -12 h	13.24±2.7	70.34±2.7	94.93±0.2
FDE -24h	13.67±2.6	72.61±2.8	97.1±0.1
FDE -48h	13.77±2.6	73.18±2.8	97.67±0.1
FDE -72h	13.99±2.5	74.32±2.9	98.91±0.1
Vitamin C	21.63	100	100

Values represent means ± SD (n=3).

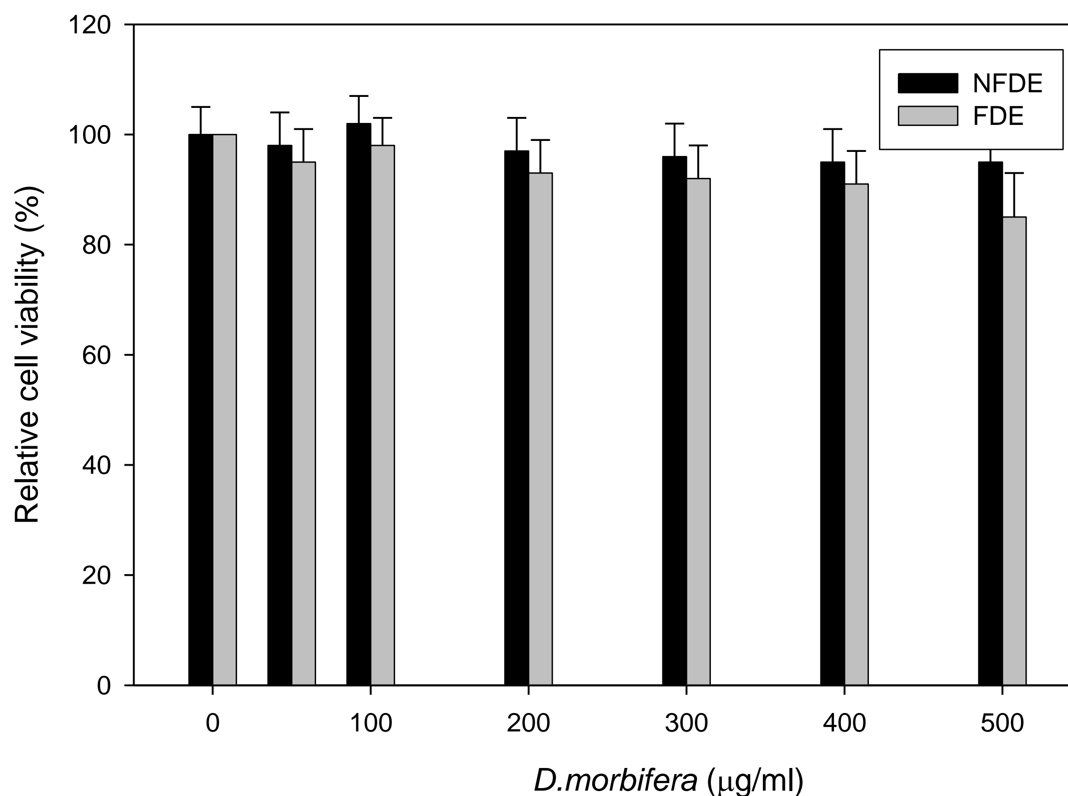

Figure 1. Effects of NFDE and FDE on viability of 3T3-L cells. Cells were seeded at a concentration of 1 × 10^4 cells/well in a 96-well plate and differentiation allowed for 4 days following treatment with a range of concentrations of NFDE and FDE. Following harvesting, cytotoxicity was determined with the MTT assay. Results are presented as means±SD of experiments performed in triplicate.

DISCUSSION

In this study, the functional components of *D. morbifera* fermented with lactic acid bacteria was investigated. The antioxidant effect of the fermented extracts according to the lactic acid bacteria was analyzed. Polyphenols, originally known as Vitamin P, have various potential health benefits. Polyphenol compounds are widely distributed in medicinal plants. Several physiological properties of these phytochemicals have been reported, including antioxidant and anticancer activities (Liu, 2004; Manach et al., 2005, Kang et al., 2011). Notably, at 72 h of fermentation, the total polyphenol content of FDE (640.03 μg/ml) was higher than that of NFDE (562.44 μg/ml) (Table 1). During fermentation, a number of enzymes, such as protease, amylase and lipase, are secreted, leading to increased levels of phenolic substances and consequently, elevated antioxidative activity (Manach et al., 2005). The increase in polyphenol content was attributed to an increase in the quantity of

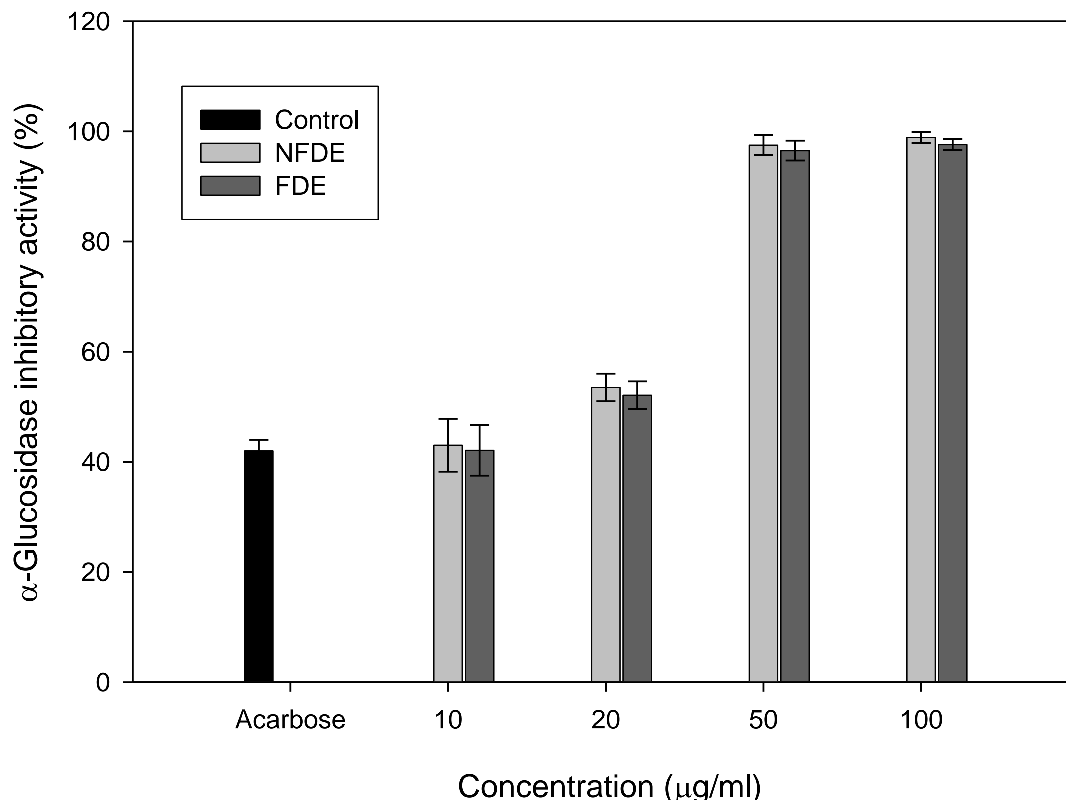

Figure 2. Inhibitory effects of NFDE and FDE on α-glucosidase activity. Results are presented as means±SD of experiments performed in triplicate. Acarbose (50 μg/ml) was used as a positive control.

phenolic compounds. Phenolic substances impart a unique color to plants and are significantly involved in determining taste. The antioxidative compounds obtained from natural products to date have mainly been identified as phenolic compounds and flavonoids. In particular, caffeic acid, chlorogenic acid and gentisic acid exert strong antioxidative effects (Chung 1999).

The DPPH radical scavenging activities of NFDE and FDE increased in a concentration-dependent manner, as shown in Table 2. FDE displayed slightly higher DPPH radical scavenging activity than NFDE within the concentration range of 0.1, 1, and 5 mg/ml. The positive control group (Vitamin C) displayed significantly higher radical scavenging activity than *D. morbifera* at equivalent concentrations. In a study by Jeon et al. (2011) comparing treatment with extracts of ginseng and lactic acid-fermented ginseng (0.1 and 1.0%), activity was increased from 24.85 and 49.78% to 54.30 and 86.36%, respectively. This study concluded that fermentation of *D. morbifera* with lactic acid bacteria is possible and that is effective to increase the antioxidant effects of *D. morbifera*. Kang et al. (1995) reported enhanced DPPH radical scavenging antioxidant activity by phenolic compounds with greater reducing power. Moreover, in their study, FDE with high total polyphenol content displayed high DPPH radical scavenging activity, further supporting a correlation between phenolic compounds and DPPH radical scavenging ability. The electron donating ability via radical scavenging of DPPH contributes to the antioxidant activity of phenolic substances.

To examine the cytotoxicity of *D. morbifera* to 3T3-L 1 cells, the MTT assay was performed using various extract concentrations (0-500 μg/ml) before and after fermentation. The results are shown in Figure 1. At the highest treatment concentration of non-fermented *D. morbifera* extract (500 μg/ml), viability of 3T3-L 1 cells was 95%, indicating no significant inhibition of cell survival. At the same concentration of fermented extract, cell viability was 85%, suggesting that fermentation broth maintaining a concentration of 500 μg/ml extract can effectively enhance cell growth without inducing toxicity. As a result, all activity experiments were conducted with concentrations of up to 500 μg/ml extract.

The α-glucosidase enzyme ultimately converts poly-saccharides to monosaccharides by α-amylase. Inhibition of these enzymes results in delayed carbohydrate hydrolysis and absorption, thereby improving postprandial glucose increase. Inhibitors of α-glucosidase activity block digestion and absorption of carbohydrates in the small intestine regardless of insulin secretion, thereby reducing the side-effects of existing drugs, such

as hypoglycemia, hepatotoxicity and dysregulation of pancreatic function. Diabetes mellitus is divided into insulin-dependent and insulin non-dependent subtypes. Current treatments include control of weight and diet, along with administration of insulin, sulfonyl urea and biguanide. However, the development of effective anti-diabetic diets using natural products that do not exert side-effects remains an urgent clinical requirement. Acarbose is a typical inhibitor of α-glucosidase that has recently been developed for use in the treatment of diabetes. With a view to controlling insulin blood glucose levels in patients with type 2 diabetes, inhibition of α-glucosidase by *D. morbifera* was examined as an indicator of antidiabetic activity. As shown in Figure 2, a dose dependent inhibitory effect on α-glucosidase was observed. Administration of 50 µg/ml acarbose, currently marketed as a diabetic improver, led to 43.8% inhibition of α-glucosidase activity. Inhibitory activities of 10 µg/ml NFDE and FDE on α-glucosidase were equivalent to that of 50 µg/ml acarbose. At concentrations of 20, 50 and 100 µg/ml, NFDE and FDE exerted higher inhibitory activity than acarbose, supporting their utility as natural materials for the improvement of diabetes mellitus.

In conclusion, the antioxidant, cytophilic and α-glucosidase inhibition effects of extracts of *D. morbifera* support its utility as a potential candidate for the development of natural anti-diabetic agents with minimal side-effects.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Enhancement of saponin accumulation in adventitious root culture of Javanese ginseng (*Talinum paniculatum* Gaertn.) through methyl jasmonate and salicylic acid elicitation

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Javanese ginseng (*Talinum paniculatum*) is an herb and one of the traditional medicinal plants in Indonesia which accumulate saponin in the root organ. However, slow root growth hampers the accumulation of the compound in this plant. Therefore, *in vitro* culture of adventitious roots offers an alternative way to increase the production of saponin. Furthermore, this study was performed to investigate the effect of methyl jasmonate (MeJA) and salicylic acid (SA) on the growth and saponin content in adventitious root of Javanese ginseng. Adventitious root culture was induced on solid Murashige and Skoog (MS) medium supplemented by 10 µM IBA (indole-3-butyric acid). Subsequently, adventitious roots were subcultured into a half strength MS liquid medium with the addition of different concentrations of MeJA or SA and incubated for 5, 10 and 15 days. Despite inhibition on the root growth, saponin production increased by 1.5 and 1.3 fold upon elicitation with 0.2 mM MeJA and SA for 15 days, respectively. Thus, MeJA and SA elicitations regulate saponin biosynthesis in adventitious root culture of Javanese ginseng as a time and dose-dependent manner.

Key words: Adventitious root, elicitation, Javanese ginseng, saponin, *Talinum paniculatum*.

INTRODUCTION

Talinum paniculatum (family Talinaceae, is a succulent plant that grows up to 100-120 cm tall and characterized by a large and many-flowered terminal panicle with pink color. In Indonesia, this plant is also called Javanese ginseng as it has swelling roots similar to *Panax ginseng* root and its extract is used as a traditional medicine for multiple diseases (Manuhara et al., 2015). Investigation of root extract from Javanese ginseng resulted in the identification of triterpenoid saponin mixtures. Further characterization from the same genus resulted in the

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identification of oleanane-type saponins which is responsible for different pharmacological activities such as estrogenic, antifertility, antibacterial, antifungal, antioxidant, and cytotoxic activities (Kohda et al., 1992; Reis et al., 2015; Thanamool et al., 2013).

In the natural habitat, the root of Javanese ginseng has a slow growth rate. Generally, it takes about 2-3 years to get more than 100 g of root per plant (Manuhara et al., 2015). Therefore, *in vitro* root culture is essential for preservation of this plant, especially to support sustainable saponin production. Adventitious root culture has been used to produce wide range of secondary metabolites which have been naturally accumulated in root organ. The advantages of adventitious root culture are the root, not influenced by geotropism, fast growth of root branch and genetically stable (Murthy et al., 2016; Inabuy et al., 2017).

Methyl jasmonate (MeJA) is a widely used elicitor to induce secondary metabolite compounds in plant cell culture. MeJA and its derivatives have been proposed to be the key signaling compounds in the process of elicitation leading to the accumulation of secondary metabolites (Ramirez-Estrada et al., 2016). Salicylic acid (SA) is another elicitor that is widely studied as a stressed molecule signal to respond to the pathogen in plants (Hernández et al., 2017). Furthermore, SA was reported to enhance phytoalexin production in cell culture and root culture of several medicinal plants such as *Withania somnifera, Anisodus luridus* and *Lepechinia caulescens* (Sivanandhan et al., 2013; Qin et al., 2014; Vergara Martínez et al., 2017). Among all biotic and abiotic elicitors, MeJA and SA are the most important elicitors used as inducers of triterpene saponin production (Moses et al., 2013). Therefore, this experiment was established to compare the effect of different elicitors (MeJA and SA) in various concentrations (0.05, 0.1 and 0.2 mM) and duration of elicitation (5, 10 and 15 days) on saponin production from adventitious roots culture of Javanese ginseng.

MATERIALS AND METHODS

Adventitious root culture

Adventitious roots were induced from the shoot culture of Javanese ginseng grown on a phytohormone-free Murashige and Skoog (MS) solid medium (Murashige and Skoog, 1962) containing 30 g/L sucrose and 8 g/L agar. 20 mg of adventitious roots were then isolated and placed onto a half-strength MS solid medium supplemented with 1 μM and 10 μM of IBA (indole-3-butyric acid) (Duchefa Biochemie, the Netherlands) or NAA (1-napthaleneacetic acid) (Duchefa Biochemie, the Netherlands). Root cultures were incubated at 25±1°C in the dark. After 28 days, adventitious roots were harvested and the respective number of lateral roots that appear as well as final biomass were measured. Subsequently, the adventitious roots were subcultured in a 100 ml Erlenmeyer flask containing 15 ml of half-strength MS liquid medium for treatment with elicitors.

Effect of MeJA and SA on adventitious root growth and saponin production

Treatments of adventitious roots were performed with an inoculum of about 0.7 g fresh weight root segment in 20 ml of half-strength MS liquid medium supplemented with 10 μM IBA and 15 g/L sucrose. Different concentrations of MeJA (0, 0.05, 0.1, and 0.2 mM) and SA (0, 0.05, 0.1, and 0.2 mM) were added in the medium. The root was harvested after elicitation for 5, 10, and 15 days. After that, dry weight and saponin content were measured.

Extraction and determination of total saponin content

Roots were collected from MeJA and SA treated and non-treated samples at different incubation time. Subsequently, the dry weight was measured after drying the roots in the oven at 50°C for 24 h. All samples were stored on the desiccator until constant weight was reached.

Thirty milligram of dried sample was ground to a fine powder and dissolved in 1.5 ml of 96% methanol. The mixture was sonicated for saponin extraction using sonicator (Branson sonicator 3510) for 1 h and then centrifuged at 10,000 rpm for 10 min. Collected supernatant was used for total ginseng saponin analysis.

Total saponins were measured through spectrophotometer (Optima Japan SP3000 nano) as described by Fiallos-Jurado et al. (2016) with minor modification. The Liebermann-Burchard (LB) reagent was used for saponin quantification as it is capable of producing a light brown staining if this compound is present in a sample. The LB reagent consists of a 1:5 mixture of acetic acid and sulfuric acid, respectively. After mixing 1 ml sample solution with 3.5 ml of LB reagent, absorbance at 540 nm was measured in all samples after 20 min. A calibration curve based on oleanolic acid was used to determine the final concentration of saponin (mg/ml) in each solution. The total saponin content was calculated on the basis of dry weight.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Duncan's multiple range tests. All statistical analyses were performed at the level of P value less than 0.05 using SPSS 23.0 (SPSS Inc. USA).

RESULTS

Adventitious root cultivation

The number of lateral roots and biomass was affected by auxin type and respective concentration. In general, roots treated by IBA form whitish yellow lateral roots with numerous root hairs while those treated by NAA form brown yellowish lateral roots and also callus in their main roots. The roots formed in a medium containing NAA were also shorter and thicker than roots grown in a medium containing IBA.

The number of lateral roots that emerged from adventitious root explant varied in all treatments (Figure 1). The result showed that IBA induced the development of more lateral roots compared with control, while NAA showed a contrasting result. Furthermore, there was no significant difference between the roots induced by 1 μM

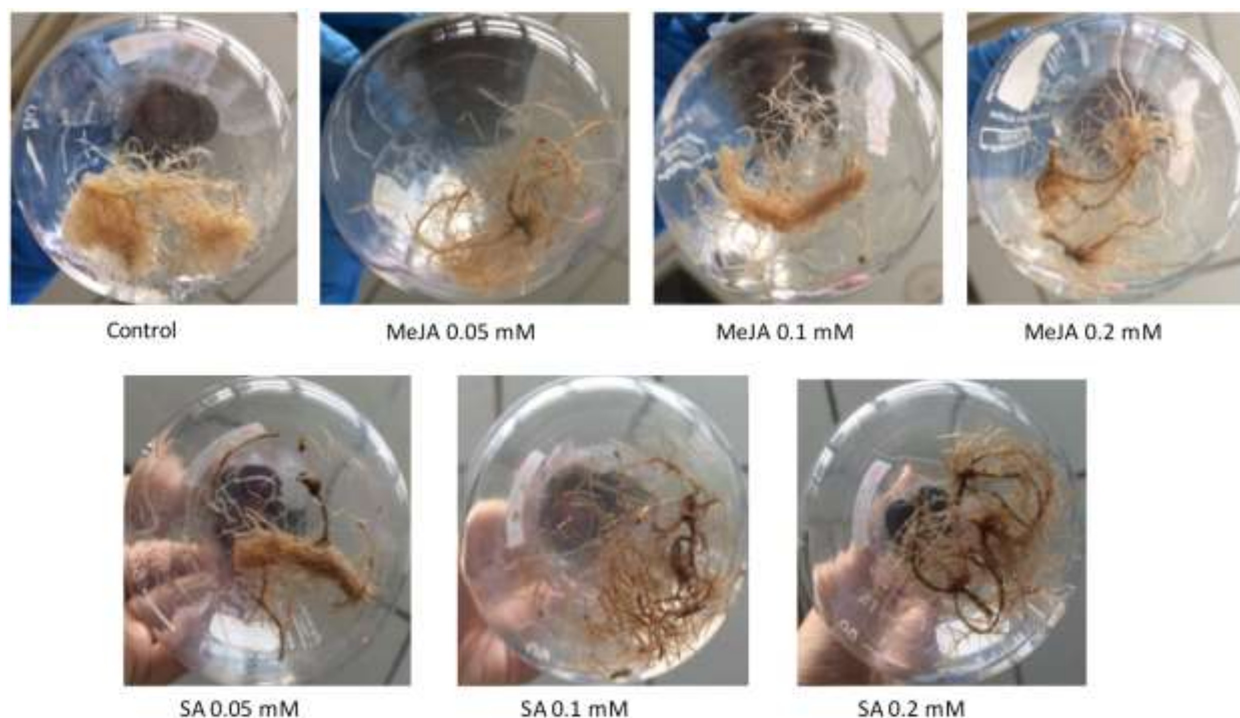

Figure 1. Morphology of adventitious roots of Javanese ginseng after treatment with different elicitors for 15 days.

Table 1. Effect of the type and auxin concentrations on the induction of lateral root and root biomass of Javanese ginseng after 4 weeks of culture.

Treatments	Concentration (µM)	Number of lateral root	Root biomass (mg)
Control	0	15.67 ± 0.58^{b}	30.069 ± 0.002^{bc}
IBA	1	23.00 ± 3.00^{a}	30.072 ± 0.001^{b}
	10	24.33 ± 4.16^{a}	30.075 ± 0.001^{a}
NAA	1	9.00 ± 4.36^{c}	20.068 ± 0.001^{c}
	10	4.00 ± 1.00^{c}	20.067 ± 0.001^{c}

Values were presented as mean ± standard deviation using five replicates. Values with same alphabets are significant at $p<0.05$ (one-way ANOVA) according to Duncan Multiple Range test.

and 10 µM IBA in terms of the number of lateral roots, yet 10 µM IBA resulted in the highest root biomass (Table 1). The adventitious roots incubated in 10 µM IBA has growth rate of 0.035 g/day with doubling time for 27 days. Therefore, IBA 10 µM was further added to the medium for adventitious root cultivation.

Effect of MeJA and SA on adventitious root growth

Both treated and non-treated roots were growing on the liquid MS medium. However, the treated roots showed a slower growth rate compared to non-treated control. Furthermore, the color of treated roots changed and tends to be darker compared to that in control (Figure 1). Dry weight of adventitious root culture during the 5, 10, and 15 days after treatment with various concentrations of MeJA and SA are shown in Figure 2. In each incubation time, root biomass decreased from 32-37% when treated with MeJA. Similar result was also observed on SA treated samples in which root biomass decreased from 23 to 35%.

Effect of MeJA and SA elicitation on saponin production

Increased concentration of MeJA and SA resulted in the

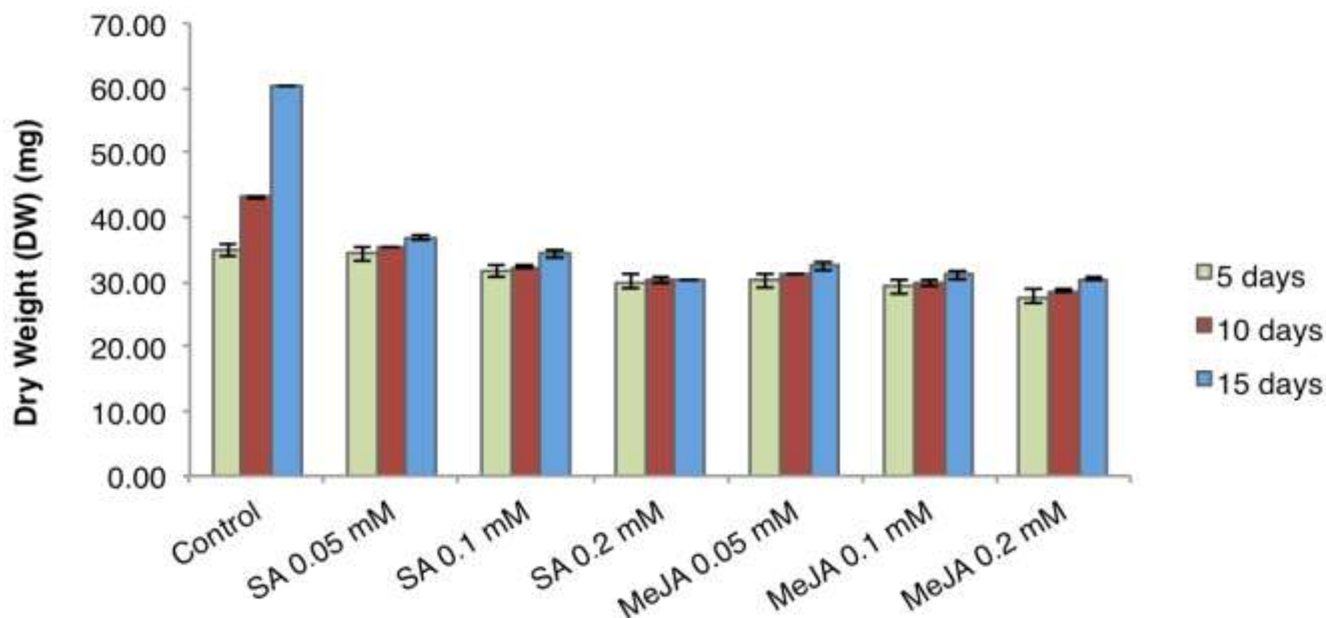

Figure 2. The effect of various concentrations and durations of MeJA and SA on adventitious root growth.

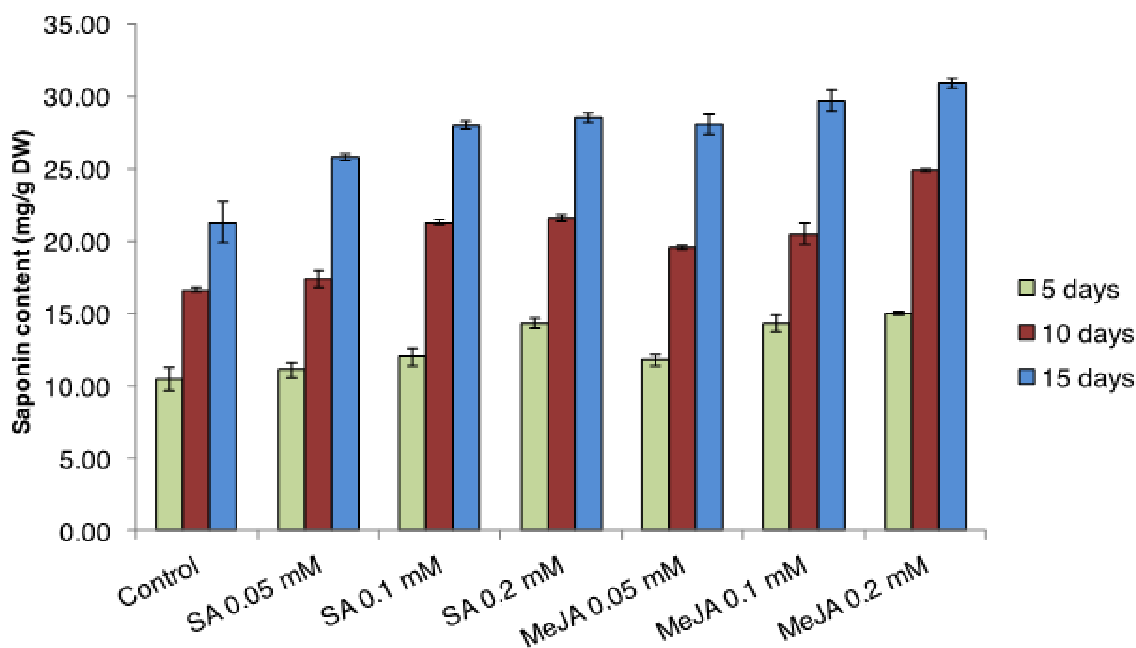

Figure 3. The effect of various concentrations and durations of MeJA and SA on saponin content in adventitious roots.

accumulation of saponin content in the adventitious roots (Figure 3). The longer the incubation period which allow contact with elicitors, the more there is saponin accumulation in the root culture. At the 5-day treatment, saponin content increased as long as there is increase in elicitor concentration; however, the results slightly increase. This is because during the 5 days incubation, the roots are still in the adaptation stage with the new

medium. At the 10-day treatment, saponin content at the root also increase with increasing elicitor concentration of 0.2 mM of MeJA and SA increased saponin content of about 1.4 and 1.2 times respectively compared to control.

Amount of total saponin increased with increasing MeJA and SA concentrations, and reached a maximum accumulation up to 30 mg/g dry weight (DW) upon treatment with 0.2 mM MeJA for 15 days. In every treatment compared to control, MeJA with concentration of 0.05, 0.1 and 0.2 mM increased saponin content by 1.2, 1.3 and 1.5 fold, respectively, whereas SA with concentration of 0.05, 0.1 and 0.2 mM increased saponin content by 1.1, 1.26 and 1.3 fold, respectively.

DISCUSSION

In vitro tissue cultures have been selected as alternatives for the production of desired saponin compounds in many plants (Espinosa-Leal et al., 2018; Leonard et al., 2018). These techniques could solve different problems associated with saponin extraction from natural-grown plants as well as offer a more sustainable mean for the production of this secondary compound. Furthermore, some plant secondary compounds are controlled in a tissue-specific manner, thus root or shoot culture are more preferred as the production vehicles (Faizal et al., 2013). Taken together, plant tissue culture could help to facilitate the improvement of saponin production through biotechnological approaches.

In this study, adventitious root culture was induced from Javanese ginseng by employing different concentrations of IBA and NAA. IBA was found to be more superior over NAA which may be due to the effects of IBA on the induction of lateral root. In morphological and histological aspects, the roots formed under IBA treatment developed normally compared to NAA. Based on the study conducted by Pacurar et al. (2014), growth regulators such as IBA have a higher potential to induce roots than other auxin hormones. The study also reported that IBA has a prominent effect on the stimulation of *in vitro* lateral roots from a mature root tissue. Similarly, adventitious roots of *P. ginseng* with IBA-containing medium were more effective in inducing lateral root and root growth compared to NAA (Murthy and Paek, 2016).

Many important plant secondary metabolites are hindered by low production titer which could prevent their commercial application. One of the technical approaches used to overcome this problem is application of elicitor to trigger a physiological response in plants and results in the accumulation of secondary metabolites. Moreover, elicitation depends on many factors such as elicitor type and concentration, the growth stage of the culture at the time of elicitor addition and incubation period with elicitor (Ramirez-Estrada et al., 2016). In this context, we used different concentrations of MeJA and SA together with different incubation periods to improve saponin production in adventitious roots of Javanese ginseng.

Both treatment of MeJA and SA induced a negative growth effect on adventitious roots of Javanese ginseng, which is a common phenomenon observed in plants. MeJA is suggested to inhibit root growth by interfering with transmembrane auxin flux pathway (Yan et al., 2016). Likewise, it has been reported that root growth in Arabidopsis was decreased upon modulation with SA signaling pathway (Rivas-San Vicente and Plasencia, 2011). Similarly, the elicitation using MeJA and SA on *P. ginseng* adventitious root culture showed inhibition of the root growth up to 25% (Ali et al., 2006) as reported in other plants (Saini et al., 2013; Eichmann and Schäfer, 2015).

The different growth shown by the treated roots indicate a stress condition. SA as a phenolic compound was produced naturally on every plant, and accumulation of phenolic compound on roots might occur as indicated by browning in response to additional SA put into the medium. In view of the inverse relationship between the production of biomass and the accumulation of the secondary metabolite, the root growth depression arising from elicitation may favor the synthesis of the secondary metabolite.

Amount of total saponin in the adventitious root cultures of Javanese ginseng increased with increasing MeJA or SA concentrations, and reached a maximum following the addition of 0.2 mM MeJA for 15 days to the cultivation medium. Previous study in whole root culture of *G. glabra* showed that 2 mM MeJA and 1 mM SA increased respective glycyrrhizin production by 3.8 and 4.5 fold after elicitation for 24 h (Shabani et al., 2009); whereas, adventitious root culture of *P. ginseng* required 7 days incubation with 0.2 mM of MeJA or SA for increasing the production of ginsenoside to 4 and 3 fold, respectively (Ali et al., 2006). The prolonged elicitation for 35 days in root culture of *Centella asiatica* significantly increased the accumulation of saponin by 4 to 6 fold (Mangas et al., 2008). The result indicated that the optimum concentration of signaling compounds varies according to the species. Also, contact time with elicitor was an important factor for enhanced production of secondary metabolite. In conclusion, MeJA and SA are potentially used to improve the yield of saponins in adventitious root cultures in Javanese ginseng.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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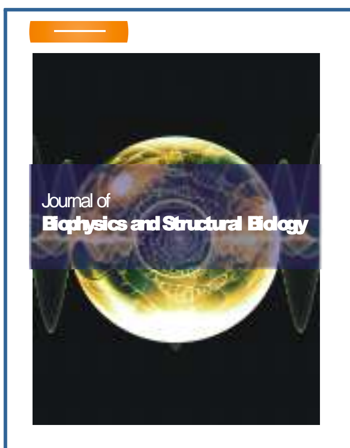

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