

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 μ g/ml. Both NFDE and FDE (100 μ g/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 moribifera* has been increasingly cultivated on Jeju Island and some regions of the Korean coastline along the southwestern sea. *D. moribifera*, 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. moribifera* have been documented, such as improvement of lipid abnormalities, diabetic disease, immune activity, thrombosis and kidney loss 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. moribifera*.

Most of the foods using *D. moribifera* 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. moribifera* extract. This study focused on evaluation of the antioxidant, cell toxicity and anti-diabetic activities, in particular, alpha-glucosidase activity of *D. moribifera* distilled water extracts. Our results may serve as a platform to evaluate the utility of *D. moribifera* extracts as a nutraceutical source for management of

diabetes in the future.

MATERIALS AND METHODS

Preparation of *D. moribifera* extracts

Boughs of *D. moribifera* 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. moribifera* boughs were cut into 1.0 cm length sections. The distilled water extract of *D. moribifera* (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. moribifera* (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 $1-5 \times 10^7$ CFU/ml. *D. moribifera* 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 μ l), distilled water (3 ml), 250 μ l Folin-Ciocalteu's reagent solution, and 7% NaCO₃ (750 μ l) was vortexed and incubated for 8 min at room temperature, followed by dilution into 950 μ l 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 (μ g GAE/ml sample) based on a gallic acid calibration curve. The linear range of the calibration curve was 10 to 200 μ g/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 μ M 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 μ l NFDE and FDE at a range of concentrations (0, 50, 100, 200, 300, and 400 μ g/ml) and incubated at 37°C for 4 h in 5% CO₂. Cell viability was determined according to the protocol provided by the manufacturer. MTT reagent (20 μ l) 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. morbilifera*

Variable	NFDE		FDE		
	0 h	12 h	24 h	48 h	72 h
Polyphenol ($\mu\text{g GAE/ml}$)	562.44 \pm 13.2	605.75 \pm 14.2	625.30 \pm 14.7	630.21 \pm 14.8	640.03 \pm 15

Fermentation time at 37°C, GAE: gallic acid equivalent. Values represent means \pm 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 $\mu\text{g/ml}$) 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:

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100$$

where, A_s represents absorbance of the test sample and A_c the absorbance of control.

Statistical analysis

All data are presented as mean \pm STD. Differences among treatments were assessed by analysis of variance (ANOVA), followed by Dunnett's test. *p* values \leq 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. morbilifera*. Following fermentation, total phenolic contents and antioxidant activities of fermented *D. morbilifera* 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 $\mu\text{g/ml}$, 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. morbilifera* 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. morbilifera* 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 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$).

α -Amylase inhibitory activity

This study examined the inhibitory activity of *D. morbilifera* extract against α -amylase to evaluate the availability of *D. morbilifera* 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 $\mu\text{g/ml}$ and 42.1, 52.1, 96.5, and 97.6% at FDE concentrations of 10, 20, 50, and 100 $\mu\text{g/ml}$, 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).

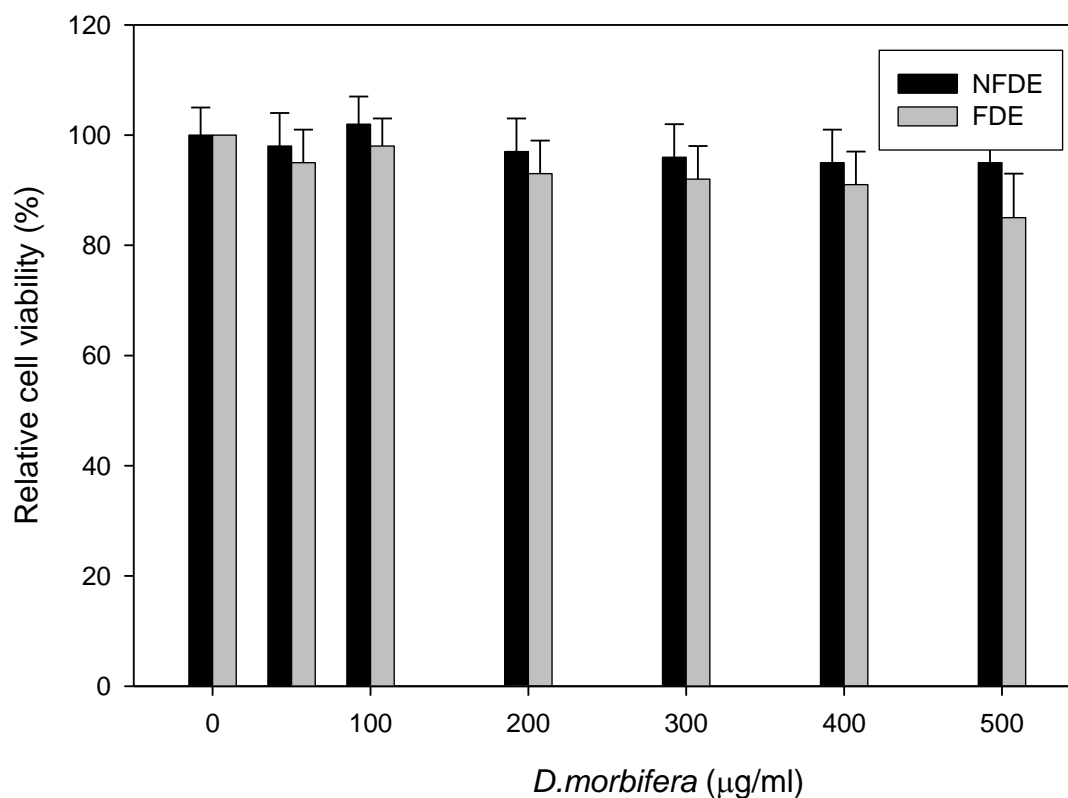


Figure 1. Effects of NFDE and FDE on viability of 3T3-L cells. Cells were seeded at a concentration of 1×10^4 cells/well in a 96-well plate and differentiation allowed for 4 days following treatment with a range of concentrations of NFDE and FDE. Following harvesting, cytotoxicity was determined with 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

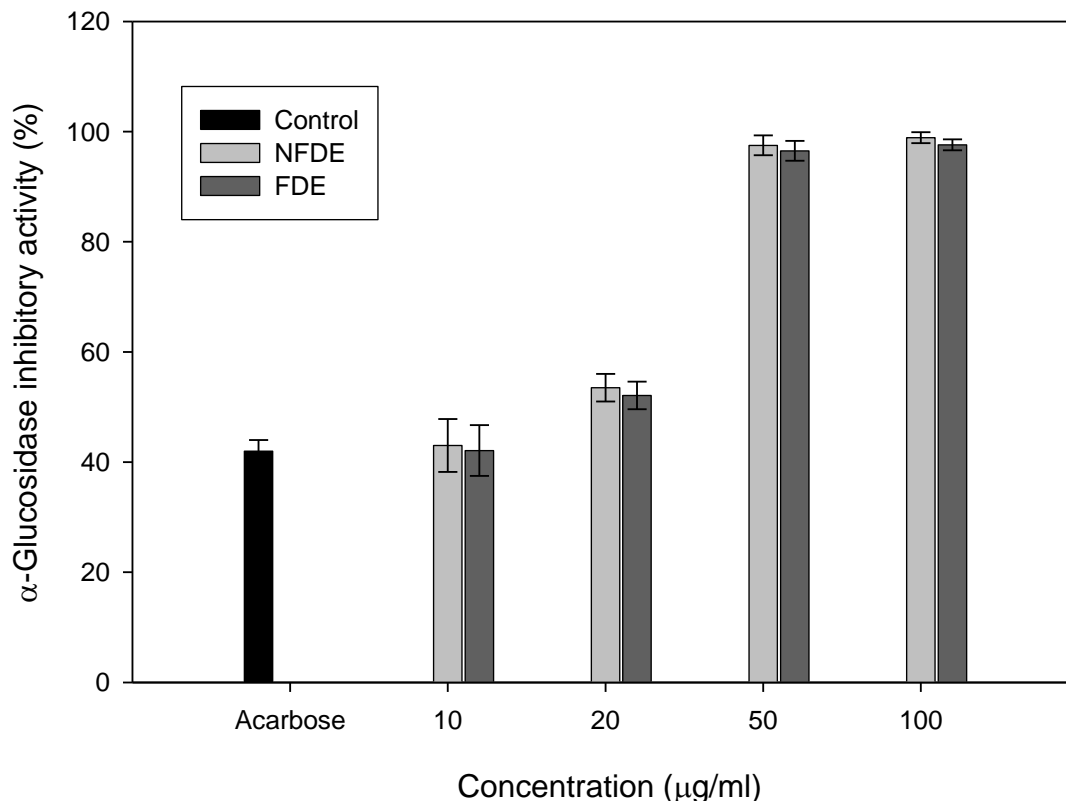


Figure 2. Inhibitory effects of NFDE and FDE on α -glucosidase activity. Results are presented as means \pm SD of experiments performed in triplicate. Acarbose (50 $\mu\text{g/ml}$) was used as a positive control.

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 $\mu\text{g/ml}$) before and after fermentation. The results are shown in Figure 1. At the highest treatment concentration of non-fermented *D. morbifera* extract (500 $\mu\text{g/ml}$), 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 $\mu\text{g/ml}$ extract can effectively enhance cell growth without inducing toxicity. As a result, all activity experiments were conducted with concentrations of up to 500 $\mu\text{g/ml}$ extract.

The α -glucosidase enzyme ultimately converts polysaccharides 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. morbilifera* 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 $\mu\text{g/ml}$ acarbose, currently marketed as a diabetic improver, led to 43.8% inhibition of α -glucosidase activity. Inhibitory activities of 10 $\mu\text{g/ml}$ NFDE and FDE on α -glucosidase were equivalent to that of 50 $\mu\text{g/ml}$ acarbose. At concentrations of 20, 50 and 100 $\mu\text{g/ml}$, 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. morbilifera* 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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