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...
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 sulfonlurea, 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 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. 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 1×10⁷ 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% NaCO₃ (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 × 10⁴ 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% CO₂. 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.
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 Na₂CO₃ (0.1 M). Absorbance of released p-nitrophenol was measured at 405 nm using a multplate 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:

\[
\text{Inhibitory activity} (\%) = \left(1 - \frac{A_s}{A_c}\right) \times 100
\]

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

<table>
<thead>
<tr>
<th>Variable (μg GAE/ml)</th>
<th>NFDE</th>
<th>FDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>562.44±13.2</td>
<td>605.75±14.2</td>
</tr>
<tr>
<td>12 h</td>
<td>625.30±14.7</td>
<td>630.21±14.8</td>
</tr>
<tr>
<td>24 h</td>
<td>630.21±14.8</td>
<td>640.03±15</td>
</tr>
<tr>
<td>48 h</td>
<td>625.30±14.7</td>
<td>630.21±14.8</td>
</tr>
<tr>
<td>72 h</td>
<td>640.03±15</td>
<td></td>
</tr>
</tbody>
</table>

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

This study examined the inhibitory activity 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 (Fig 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*

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

Values represent means ± SD (n=3).

### 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

![Graph showing relative cell viability of NFDE and FDE on viability of 3T3-L cells](image_url)

**Figure 1.** Effects of NFDE and FDE on viability of 3T3-L cells. Cells were seeded at a concentration of $1 \times 10^4$ 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.
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.
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. moriblera 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. moriblera 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.

ACKNOWLEDGEMENT

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REFERENCES