

Full Length Research Paper

Antioxidant, cytotoxic and antimicrobial activities of *Dendropanax morbifera* and sweet potato extracts for production of health-oriented food materials

KiBeom Lee¹, Ju Hyun Park² and Yun Sung Kim²

¹Bioindustry Center, Incheon Technopark, 12 Gaetbeol-ro, Yeonsu-gu, Incheon, South Korea 21999.

²Genetrone Biotech Co., Ltd, 2F, 4F, Sehyun Building, 15 Hongsanbuk-ro, Wansan-gu, Jeonju-si Jeollabuk-do, South Korea.

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The antioxidant, cytotoxic and antimicrobial effects of fermented and non-fermented extracts of 'the Korean shrub' *Dendropanax morbifera* and sweet potato were compared to assess the potential utility of these species in the development of health-oriented food. Non-fermented extract (NFDSE) was obtained from the leaves and branches of *D. morbifera* and the bodies of sweet potato using distilled water. The fermented extract (FDSE) was prepared by inoculating the above-obtained extracts with *Lactobacillus plantarum* and *Lactobacillus brevis*. The extracts of the two species combined *D. morbifera* and sweet potato exhibited substantial antioxidant activity. Moreover, NFDSE at 24 h exerted more antioxidant effects than FDSE (72.57% vs. 71.08%, respectively) at a concentration of 100 mg/ml. Comparison of the effects of the non-fermented and fermented extracts on HaCaT keratinocyte cell viability revealed that FDSE had a slightly higher cytotoxicity than NFDSE (94.8% vs. 102.7% viability, respectively) at a concentration of 500 µg/ml. It was further found that NFDSE and FDSE had the strongest antimicrobial effects against *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* (a Gram-positive coccidium). Therefore, it is obvious that extracts of *D. morbifera* and sweet potato represent 'novel candidates' for the production of functional anti-aging agents with minimal side effects.

Key words: *Dendropanax morbifera* and sweet potato, antioxidant activity, cytotoxicity, antimicrobial effects, health-oriented food.

INTRODUCTION

Due to rapid industrial developments and economic growth, research interest in health-related products and, consequently, life expectancy has greatly increased (Bergh and Nilsson, 2010). In addition, food habits have changed from the traditional form of consuming fermented foods to western diets. Excessive intake of

calories and fat via meat-based diets and high quantities of processed food and food additives is associated with a higher incidence of chronic degenerative disorders, such as hypertension, stroke, diabetes, cancer, macular degeneration and chronic liver disease. Accordingly, substitution of meat-based diets with vegetarian options

E-mail: klee02@empal.com Tel: +82-32-260-0831; Fax: +82-32-260-0897.

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is the current diet trend. In addition, there is an increased tendency to ingest food extracts prepared from physiological components of vegetable material. A representative example is plant-derived fermented liquid consumed in large quantities in the private sector over recent years. Following age-induced fermentation of plant material, many components are activated and converted into forms that are easy to absorb. Fermented liquid plant extracts have been shown to exert multiple beneficial effects through regulatory functions along with antioxidant, anti-obesity and anti-cancer activities (Kim et al., 2011; Lee et al., 2012; Yang et al., 2011), although research on this topic is still in its infancy. At present, various plant materials are utilized in view of their effects on wellbeing. However, related functionalities and physiological aspects are yet to be investigated in detail, with the only knowledge so far being down to long-term experience and partial facts from word of mouth.

Recently, *Dendropanax morbifera* and sweet potatoes were suggested to be beneficial for human health. This species belongs to the genus *Dendropanax* in the family *Araliaceae*. Around 75 species of *Dendropanax* are distributed in East Asia, Malay Peninsula, Central and South America, with one species identified in Korea. This subtropical broad-leaved evergreen tree is economically important due to its utility in the production of golden varnish (Moon et al., 1999; Kim et al., 2006), and has been increasingly cultivated on Jeju Island and regions of the Korean coastline along the southwestern sea. 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, supporting its potential utility in the development of novel therapeutic drugs and functional materials.

Sweet potatoes are one of the most common seasonal foods and widely used as a major food resource along with cereals, such as rice and barley. Sweet potatoes mostly constitute starch, along with water and β -carotene, and are rich in minerals and dietary fiber. Research to date has focused on processing of foods from sweet potato, such as chips and noodles, and the techniques involved. However, to our knowledge, no studies have explored processing of beverages from sweet potato tubers.

The properties of fermented sugar extracts of *D. morbifera* and sweet potato are not known although the functions of specific raw materials and extracts have been investigated. Both *D. morbifera* and sweet potato

are known to contain various physiologically active substrates but have rarely been used in processed foods so far. The main objective of the current study was to examine the potential utility of *D. morbifera* and sweet potato distilled water extracts fermented with the aid of lactic acid bacteria in health-oriented food products. Hydrothermal extraction and various *in vitro* experiments were applied to obtain a functional beverage from *D. morbifera* and sweet potato extracts with antioxidant, cytotoxic and antimicrobial activities.

MATERIALS AND METHODS

Preparation of *Dendropanax morbifera* and sweet potato extracts

Boughs of *D. morbifera* and sweet potato were collected from a natural habitat in South Korea, Jeju Island in February 2018. Samples were dried at room temperature and subjected to the extraction process. Collected boughs were cut into 1.0 cm sections. Sweet potatoes were finely pulverized and stored at a temperature of -20°C . The distilled water extract of *D. morbifera* and sweet potato (NFDSE) was obtained using 20 volumes of water at 95°C for 4 h. Fermented *D. morbifera* and sweet potato extract (FDSE) was prepared as follows: *Lactobacillus plantarum* and *Lactobacillus brevis* strains 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*. For fermentation, the *D. morbifera* and sweet potato solution (5%) was inoculated with fresh bacterial subculture (4% v/v) at 37°C for 24 h, followed by sterilization and filtration. The filtered solution of fermented sample was concentrated and spray-dried.

Measurement of antioxidant activity of the 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 1, 10 and 100 mg/ml, with vitamin C (Vit. C) treatment used as the positive control. After incubation 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 the extracts

HaCaT keratinocytes obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea) were employed 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 1.0×10^5 cells per well. Next, cells were treated with 200 μl NFDSE or FDSE at a range of concentrations (10, 50, 100, 200, 300, and 500 $\mu\text{g/ml}$) at 37°C for 24 h in 5% CO_2 . Cell viability was determined according to the manufacturer's protocol. MTT reagent (20 μl) was added to individual wells and incubated under similar conditions for 1 h and absorbance 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.

Table 1. DPPH radical scavenging activity (%) of distilled water and fermented extracts of *D. morbifera* and sweet potato.

Sample	Concentration (mg/ml)		
	1	10	100
NFDSE	4.13±0.54	25.54±2.8	72.57±4.4
FDSE - 24 h	3.27±0.44	24.17±2.8	71.08±4.6
Vitamin C	21.63	100	100

Values represent means ± SD (n=3). Means not sharing a common letter were significantly different at $p < 0.05$.

Antimicrobial activity measurement

Gram-positive *Staphylococcus epidermidis*, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* and Gram-negative *Bacillus cereus* and *Escherichia coli* ATCC 26922 strains used for the antibacterial assay were purchased from the Korean Society of Microbiology. Nutrient broth and agar were used for culture of microorganisms. For measurement of antibacterial activity, growth medium containing 1.5% agar was spread on the bottom of a petri dish, followed by growth medium containing 0.6% agar, to prepare a 2-fold plate culture medium. After adding a dosage of 70 μ l dosages of 100% extracted solution of NFDSE or FDSE to a 0.8 cm diameter disc, the strain was placed on a flat plate covered with growth medium and incubated at 37°C for 24 h to measure the growth inhibitory effect. Tetracycline and streptomycin as the standard drug were used in the experiment.

Statistical analysis

All data are presented as mean ± standard deviation of three replicates. Differences among treatments were assessed by analysis of variance (ANOVA), followed by Dunnett's test. p -value of < 0.05 was regarded as significant.

RESULTS

DPPH radical scavenging activity of *D. morbifera* and sweet potato extracts

The scavenging activities of NFDSE and FDSE for DPPH radicals increased with the treatment concentrations (Table 1). NFDSE exerted increasing inhibitory effects (4.13, 25.54, and 72.57%) at concentrations of 1, 10 and 100 mg/ml, respectively. Within this concentration range, the inhibitory effects of FDSE at 24 h were 3.27, 24.17, and 71.08%, respectively, indicating that both NFDSE and FDSE possess good scavenging activity for DPPH radicals at the concentrations tested. Vitamin C, the positive control, displayed excellent scavenging ability (21.63, 100, and 100%) within the same concentration range.

Effects of *D. morbifera* and sweet potato extracts on HaCaT keratinocyte cell viability

The potential use of NFDSE and FDSE as health-oriented

food components was further ascertained based on specific bioactivities, such as cytotoxicity. To determine the effects of the extracts on cell viability, the MTT assay was performed on HaCaT keratinocyte cells treated with 10 to 500 μ g/ml NFDSE or FDSE. The results are expressed as a percentage of surviving test cells relative to the control group (Figure 1). No significant toxicity of either the fermented and non-fermented extracts were observed within the range of concentrations examined against HaCaT keratinocytes.

Influence of *D. morbifera* and sweet potato extracts on antimicrobial activity

Antimicrobial activities of NFDSE and FDSE against five bacterial strains (*S. epidermidis*, *S. aureus* ATCC 25923, *E. faecalis*, *B. cereus*, and *E. coli* ATCC 26922) were examined using an agar well diffusion method. As summarized in Table 2, gram-positive *S. epidermidis*, *S. aureus* ATCC 25923 and *E. faecalis* strains showed susceptibility to both NFDSE and FDSE at a concentration of 200 mg/ml with inhibition zone diameters of 14.3, 12.1, and 8.2 mm for NFDSE and 15.2, 13.5, and 9.3 mm for FDSE, respectively. At the same test concentrations, the two gram-negative strains, *B. cereus* and *E. coli* ATCC 26922, showed susceptibility to NFDSE and FDSE with inhibition zone diameters of 6.7 and 6.3 mm for NFDSE and 7.0 and 6.8 mm for FDSE, respectively. Our results indicate that highest inhibitory activity of the extract in both fermented and non-fermented forms is against *S. epidermidis* and the weakest activity against *E. coli* ATCC 26922. The inhibition zone diameters with streptomycin and tetracycline, and the positive controls, ranged from 11.5-19.7 mm and 12.3-20.6 mm, respectively.

DISCUSSION

Oxidative stress is defined as intracellular damage caused by an imbalance between oxidants and antioxidants. Oxidants are produced during normal metabolism *in vivo* and highly generated in pathological conditions (Sies, 1997). Oxidative stress is known to be a

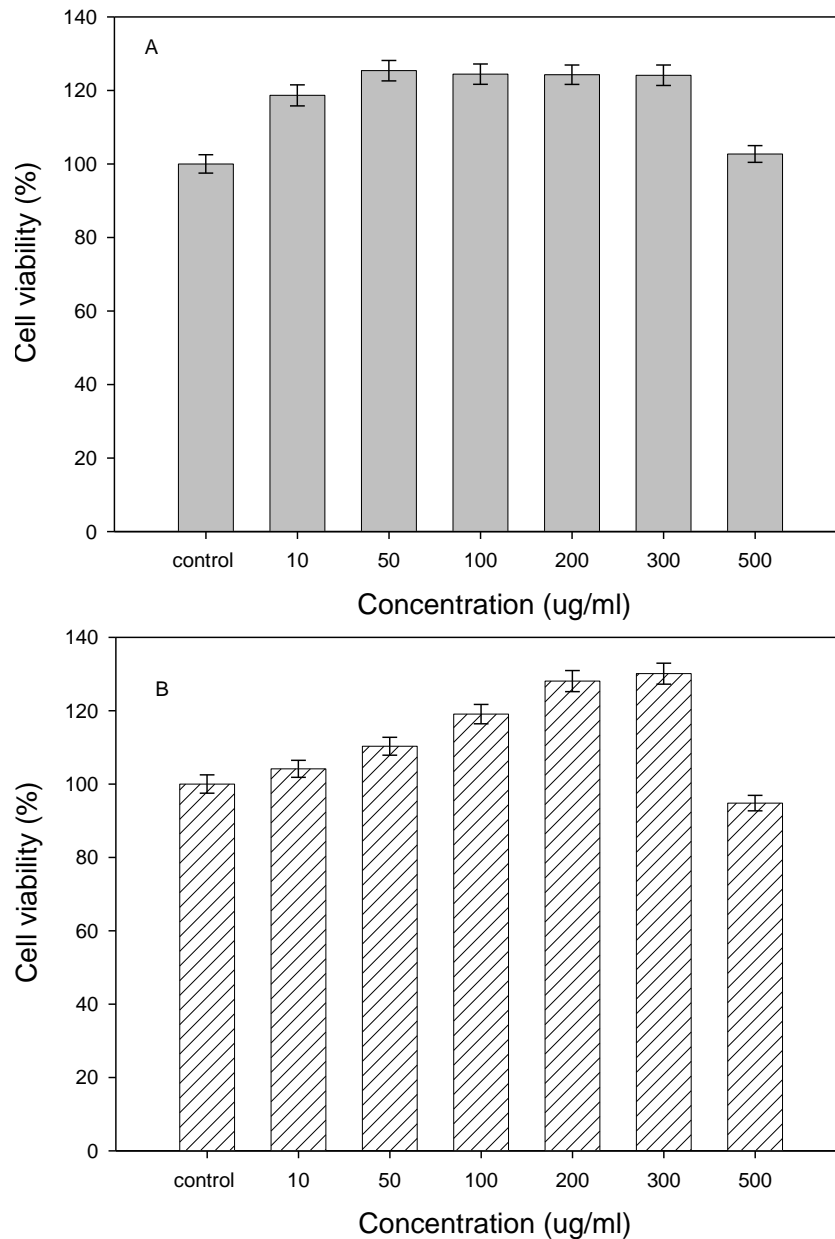


Figure 1. Effects of NFDSE and FDSE on viability of HaCaT keratinocyte cells. Cells were seeded at a concentration of 1×10^5 cells/well in a 96-well plate and differentiation allowed for 24 h, following treatment with a range of concentrations of NFDSE and FDSE. Following harvesting, cytotoxicity was determined with the MTT assay. Results are presented as means \pm SD of experiments performed in triplicate. (A) Non-fermented extract (NFDSE), (B) Fermented extract (FDSE).

direct cause of aging and several chronic diseases, such as cancer and arteriosclerosis, and the discovery of food-derived antioxidant agents to control these conditions is a major research concern (Finkel and Holbrook, 2000). The purpose of the current study was to evaluate the antioxidative activity of extracts of *D. morbifera* and sweet potato, which are known to contain high levels of antioxidants.

Antioxidants are capable of reducing the stable DPPH radical (purple) to its non-radical form, DPPH-H (yellow). The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating ability to ROS. In the DPPH radical scavenging assay, the antioxidant activities of NFDSE and FDSE were relatively good but still lower than that of Vitamin C, which showed excellent DPPH scavenging effects at all the concentrations examined.

Table 2. Diameters of inhibition zones of NFDSE and FDSE against selected microorganisms.

Pathogenic bacterial strain	Treatment			
	NFDSE (200 mg/ml)	FDSE (200 mg/ml)	Streptomycin (30 µg/ml)	Tetracycline (30 µg/ml)
	Clear zone (mm)			
<i>Staphylococcus epidermidis</i>	14.3±0.23	15.2±0.32	19.7±1.12	20.6±0.32
<i>Staphylococcus aureus</i> ATCC25923	12.1±1.05	13.5±1.05	18.1±0.23	19.3±1.03
<i>Enterococcus faecalis</i>	8.2±0.25	9.3±0.67	16.9±1.16	17.7±1.32
<i>Bacillus cereus</i>	6.7±0.25	7.0±0.25	11.8±1.05	12.5±1.07
<i>Escherichia coli</i> ATCC 25922	6.3±0.23	6.8±0.25	11.5±1.05	12.3±1.05

Treatment groups: NFDSE, non-fermented extract; FDSE, fermented extract. Values represent means ± SD (n=3). Means not sharing a common letter were significantly different at $p < 0.05$.

The electron donating ability was evaluated as an index of antioxidant activity against phenolic acid, flavonoids and other phenolic substances, whereby higher reducing power was correlated with greater electron donating ability (Kang et al., 1995). This result is consistent with previous studies showing that the *D. morbifera* and sweet potato extracts showed the presence of antioxidants and the ability to scavenge free radicals (Hyun et al., 2013; Zou et al., 2012; Ghasemzadeh et al., 2012).

To investigate cytotoxicity, HaCaT keratinocyte cells were treated with different concentrations of *D. morbifera* and sweet potato extracts and the MTT assay was performed. Cell proliferation was observed at a range of extract concentrations (10 to 300 µg/ml), indicating weak toxicity or a cell protective effect. Optimal proliferation was detected at a concentration of 300 µg/ml NFDSE and FDSE, following which proliferative ability was decreased and toxicity evident.

Our experiments disclosed antimicrobial activity of NFDSE and FDSE against all five selected microorganisms, indicating relatively broad-spectrum antibacterial effects. Among these, highest antibacterial activity was observed against gram-positive *S. epidermidis* and *S. aureus* ATCC 25923 and lowest activity against gram-negative *E. coli* ATCC 26922, indicating greater resistance of gram-negative bacteria to antibacterial agents. One potential explanation for this difference in antibiotic resistance properties is variations in the cell wall structure between gram-positive and gram-negative bacteria (Epand et al., 2016).

Conclusion

This study has demonstrated antioxidant, cytotoxic and antimicrobial activities of extracts of *D. morbifera* and sweet potato. The antioxidant *D. morbifera* and sweet potato, indicating a high activity to remove harmful free radicals, have preventive and inhibitive effect on various diseases and aging. Our collective results support the utility of *D. morbifera* and sweet potato extracts as a future source of natural anti-aging and therapeutic agents with minimal side-effects.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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