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Antibacterial and anti-obesity effects of *Abeliophyllum distichum* Nakai: an *in vitro* study

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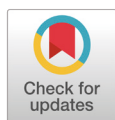
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Abstract

Interest in research on various medicinal plants has increased globally over the last few decades, possibly due to their possible antibacterial and antioxidant activities. The present study was conducted to verify the antioxidant effects, antibacterial activity, and collagen synthesis and cell viability outcomes of adipocytes upon exposure to *Abeliophyllum distichum* Nakai (AdN). Antibacterial activity was measured through the Disc diffusion method to compare the growth ability of pathogenic microorganisms (*E.coli*, *Salmonella*). The absorbance was measured at 560 nm to calculate the active oxygen scavenging ability. Fibroblasts were dispensed in a 96-well plate at a density of 1×10^5 cells-well⁻¹. The amount of procollagen was measured in each case using a procollagen type 1 C-peptide EIA KIT. The cytotoxicity of the *Abeliophyllum distichum* Nakai extract against animal adipocytes (Hanwoo backfat cells) was determined using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, a method that measures the conversion of MTS to Formazan by means of mitochondrial dehydrogenases. The concentrations of the samples were made to be 0.0125, 0.025, 0.05, 0.1, and 0.5% and all were completely absorbed into the disc in an incubator at 37°C for 24 to 36 hours. For the 0.125 mg·disc⁻¹, effects of *Abeliophyllum distichum* Nakai on the antioxidant effect, antibacterial activity, and cell viability of adipocytes were found. However, *Abeliophyllum distichum* Nakai had no effect on collagen synthesis, thus suggesting that AdN extracts may be useful for the prevention and/or treatment of obesity.

Keywords: *Abeliophyllum distichum* Nakai, adipocytes, antibacterial effect, antioxidant, collagen synthesis



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Introduction

Interest in the study of various medicinal plants has increased globally during last few decades, may be due to their antibacterial and antioxidant activities (Chew et al., 2012; Farjana et al., 2014). Plants produce secondary metabolites that possess effective pharmacological activity (Raja and Sreenivasulu, 2015). Phytochemicals, derived from secondary metabolites, help controlling active oxygen in the body through pharmacological actions including antioxidant and anti-inflammatory effects (Asensi

et al., 2011). The most important of these bioactive compounds are flavonoids, tannins, alkaloids, and phenolic compounds (Woo et al., 2001). Thus, various experiments using natural products are being conducted in many foods, cosmetics, and pharmaceutical industries (Oh et al., 2002; Hassimoto et al., 2005; Kim et al., 2007).

Abeliophyllum distichum Nakai (AdN), a Korean native plants, is known to exhibit various effects on the leaves (Xiong et al., 1996; He et al., 2000). Especially, it has been reported that the antioxidant effect and antibacterial effect are extraordinary due to the large amounts of polyphenol and flavonoid (Ji et al., 1993; Ko et al., 2019). Also, some researchers reported that the extract of AdN has the effect of improving skin wrinkles, skin aging, and whitening (You et al., 2004; Kim and Lee, 2015; Jang and Park, 2017; Chang et al., 2018). Due to effect of AdN, various industries are trying to develop products using AdN. But there is a lack of scientific evidence and research. Therefore, the objectives of this study were: (1) verification of antibacterial and antioxidative effects of AdN extract; (2) verification of the collagen and adipocyte proliferation effect of AdN extract.

Materials and Methods

Plants materials

AdN extract was supplied by Our Tree Farming Association (Geosan, Korea). Foreign matter and soil derived from the leaves of the AdN were washed with distilled water and then dried to retain a moisture content of less than 15% using an agricultural product dryer at 40°C. Dried AdN leaves were immersed and extracted for 15 minutes at 121°C and 1.2 atm in an autoclave.

Antibacterial activity

Antibacterial activity was measured using the disc diffusion method to compare the growth of pathogenic microorganisms (*E.coli*, *Salmonella*). A colony of each strain was immersed in liquid medium, activated for 24 - 36 hours and subcultured three times. Bacteria were cultured to an optical density (OD) of 0.9 at 660 nm, suspended at a concentration of 1×10^6 , and plated on agar medium and with a paper disc (6 mm, Toyo Roshi Kaicha Ltd., Tokyo, Japan). The sample concentrations were 0.0125, 0.025, 0.05, 0.1, and 0.5%, which were absorbed completely into the disc following incubation at 37°C for 24 to 36 hours. The antibacterial activity was evaluated by measuring the diameter of the clear zone (clear zone: -, no inhibition; +, very slight inhibition (6 - 10 mm); ++ slight inhibition (10 - 15 mm); +++ moderate inhibition (15 - 20 mm); and ++++, clear inhibition (15 - 20 mm)).

Scavenging ability of active oxygen

Oxidation of xanthine to uric acid by xanthine oxidase generated free active oxygen. Nitro blue tetrazolium (NBT) was reduced to formazan by active oxygen, and the absorbance was measured at 560 nm using a UV spectrophotometer (Shimadzu, Kyoto, Japan) (Aruoma et al., 1993). After adding 690 μ L of 0.1 M potassium phosphate buffer (pH 7.8) and 100 μ L of 10 mM xanthine and 5 mM NBT solution each, a 10 μ L sample of a specific concentration and 100 μ L of 0.3 units·mL⁻¹ xanthine oxidase solution were added to initiate the reaction. The products were left for 10 minutes at room temperature and the absorbance at 560 nm was measured to calculate the active oxygen scavenging ability.

Collagen proliferation

The cytotoxicity of AdN extract against human dermal fibroblasts was determined via 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, based on the conversion of MTS to formazan by mitochondrial dehydrogenases. Human dermal fibroblasts (1.0×10^5 cells·well⁻¹) were dispersed into a 96-well plate, followed by the addition of AdN extract for 18 hours according to the concentration, and incubation for 72 hours. Following the addition of 20 μ L of MTS solution, and incubation for 4 hours in a CO₂ incubator, the absorbance was measured at 450 nm. Fibroblasts were dispensed in a 96-well plate at a density of 1×10^5 cells·well⁻¹. The amount of procollagen was measured by procollagen type 1 C-peptide EIA KIT (MK101, Takara, Japan).

Adipocytes

The cytotoxicity of AdN extract against animal adipocytes (backfat cells of Hanwoo cattle) was determined using the MTS assay, to measure the conversion of MTS to formazan by mitochondrial dehydrogenases. Human dermal fibroblasts (1.0×10^3 cells·well⁻¹) were dispensed into a 96-well plate and treated with the AdN extract for 18 hours according to the concentration, followed by incubation for 72 hours. Following addition of 20 μ L of MTS solution, and incubation for 4 hours in a CO₂ incubator, the absorbance was measured at 450 nm.

Statistical analysis

All data were subjected to statistical analysis in a completely randomized design using mixed procedures of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) in the experimental unit. Orthogonal comparisons were conducted using polynomial regression to determine linear and quadratic effects at different graded levels of 0, 0.125, 0.025, 0.05, 0.1, and 0.5% of AdN extract. Differences among treatment means were determined using Tukey's multiple range test with a $p < 0.05$ indicating statistical significance.

Results

Antibacterial activity

In the concentration of 0.125 - 0.250 mg·disc⁻¹, AdN extract showed higher antibacterial activity than other treatment groups. While the antibacterial activity against *E.coli* and *Salmonella* was verified at a concentration of 0.5 - 1.0 mg·disc⁻¹, no antibacterial activity against *Salmonella* was detected at the concentration of 5.0 mg·disc⁻¹ (Table 1; Fig. 1).

Table 1. Antimicrobial effects of *Abeliophyllum distichum* Nakai extracts.

| Item (n = 3) | CON | T1 | T2 | T3 | T4 | T5 |
|--|---------|------------|------------|------------|------------|------------|
| Clear zone ^z (mm) <i>E.coli</i> | 6.0 ± 0 | 15.3 ± 0.5 | 23.3 ± 1.0 | 13.9 ± 0.8 | 14.7 ± 0.9 | 15.1 ± 0.6 |
| | - | +++ | ++++ | ++ | ++ | +++ |
| <i>Salmonella</i> | 6.0 ± 0 | 20.5 ± 0.9 | 16.9 ± 0.5 | 13.2 ± 0.6 | 13.3 ± 0.4 | 6.0 ± 0 |
| | - | ++++ | +++ | ++ | ++ | - |

The values shown are the mean of three replicates.

CON, no additive; T1, 0.0125 mg·disc⁻¹; T2, 0.25 mg·disc⁻¹; T3, 0.50 mg·disc⁻¹; T4, 1.0 mg·disc⁻¹; T5, 5.0 mg·disc⁻¹.

^z Clear zone: -, no zone of inhibition; +, very slight zone of inhibition (6 - 10 mm); ++, slight zone of inhibition (10 - 15 mm); +++, moderate zone of inhibition (15 - 20 mm); +++++, clear zone of inhibition (over 20 mm).

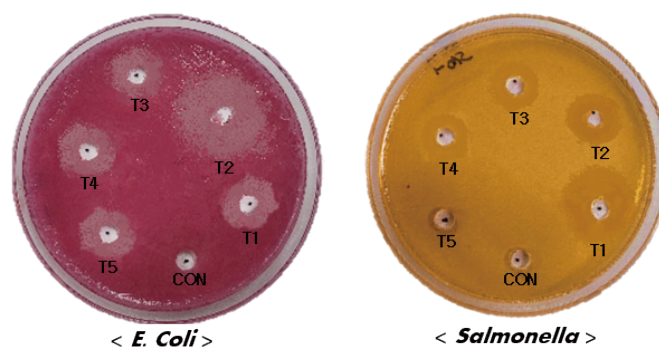


Fig. 1. Antimicrobial activities of *Abeliophyllum distichum* Nakai extract against two strains. The inoculum (about 1×10^6) was spread on the different media then, were grown at 37°C for 24 h (*E.coli*) and 48 h (*Salmonella*). The antimicrobial activity was evaluated by measuring the zone (mm) of clear zone against the test microorganisms. CON, no additive; T1, 0.0125 mg·disc⁻¹; T2, 0.25 mg·disc⁻¹; T3, 0.50 mg·disc⁻¹; T4, 1.0 mg·disc⁻¹; T5, 5.0 mg·disc⁻¹.

Measurement of scavenging ability

Allopurinol and 0.125 mg·mL⁻¹ of AdN extract showed higher active oxygen scavenging activity compared to the treatment group ($p < 0.05$), and 5.0 mg·mL⁻¹ of AdN extract showed lower than the concentration of 0.25 - 1.0 mg·mL⁻¹ (Fig. 2).

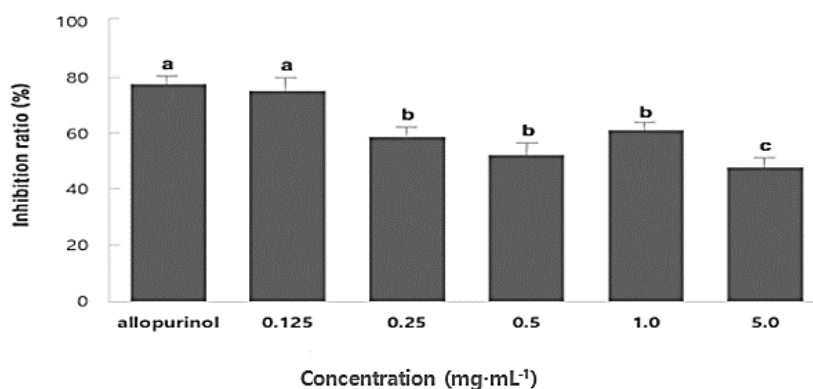


Fig. 2. Antioxidant effect of *Abeliophyllum distichum* Nakai extract. a - c: Means with the different letters in the column are significantly different ($p < 0.05$) by Duncan's multiple tests.

Collagen

The Control group showed significantly higher collagen production than other treatment groups. Collagen production increased linearly ($p = 0.040$) with increased AdN. AdN did not affect the morphological changes in human dermal fibroblasts. (Table 2; Fig. 3).

Table 2. Effect of *Abeliophyllum distichum* Nakai extract on procollagen biosynthesis and cell viability in human dermal fibroblast cultured.

| Item (n = 3) | CON | T1 | T2 | T3 | T4 | T5 | SE | p-value | |
|---------------------------------|--------|--------|--------|--------|--------|--------|-------|---------|-----------|
| | | | | | | | | Linear | Quadratic |
| Collagen (ng·mL ⁻¹) | 0.449a | 0.286b | 0.216b | 0.229b | 0.196b | 0.175b | 0.048 | 0.002 | 0.144 |
| MTS ^z | 2.091 | 1.704 | 1.833 | 1.965 | 1.710 | 1.522 | 0.167 | 0.102 | 0.815 |

The values shown are the mean of three replicates.

CON, no additive; T1, 0.0125%; T2, 0.25%; T3, 0.05%; T4, 0.1%; T5, 0.5%; SE, standard error.

^z Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay.

a, b: Means within a row with different letters are significantly different at $p < 0.05$.

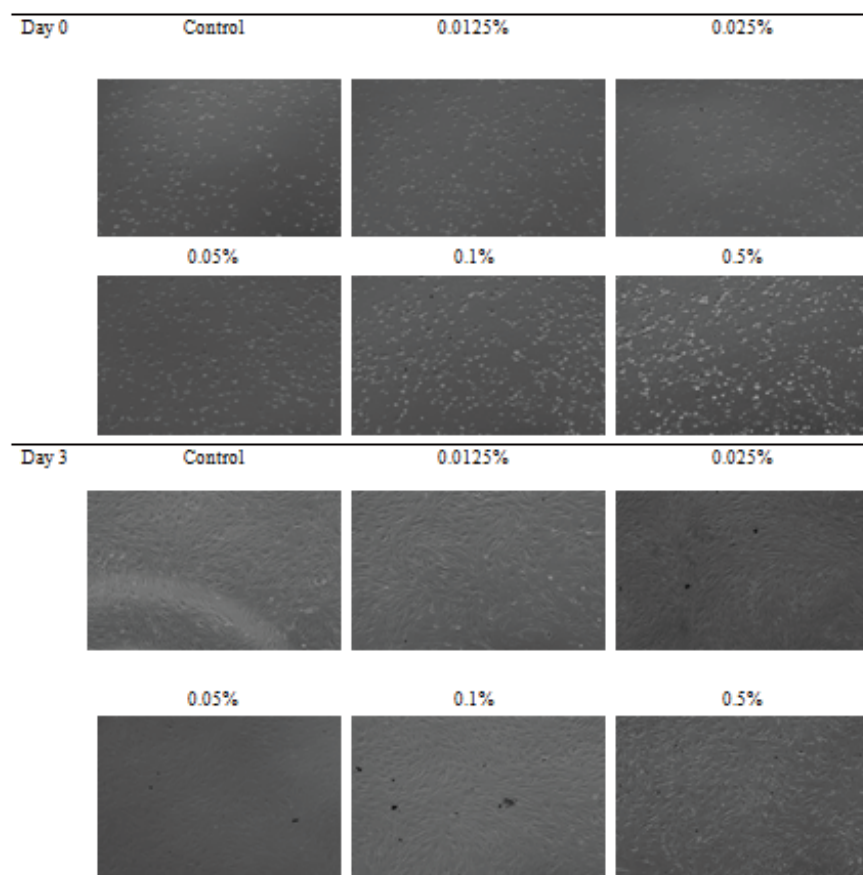


Fig. 3. Morphological comparison between human dermal fibroblast. Human dermal fibroblasts were cultured and observed by bright microscopy ($\times 100$).

Adipocyte

Viability decreased linearly ($p = 0.040$) with increased AdN (Table 3). The amount of fat cells decreased with increased AdN (Fig. 4).

Table 3. Effect of *Abeliophyllum distichum* Nakai extract on cell viability in Hanwoo backfat cell.

| Item (n=3) | CON | T1 | T2 | T3 | T4 | T5 | SE | p-value | |
|------------------|-------|-------|-------|-------|-------|-------|-------|---------|-----------|
| | | | | | | | | Linear | Quadratic |
| MTS ^z | 0.284 | 0.270 | 0.274 | 0.262 | 0.260 | 0.253 | 0.010 | 0.040 | 0.733 |

The values shown are the mean of three replicates.

CON, no additive; T1, 0.0125%; T2, 0.25%; T3, 0.05%; T4, 0.1%; T5, 0.5%; SE, standard error.

^z Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay.

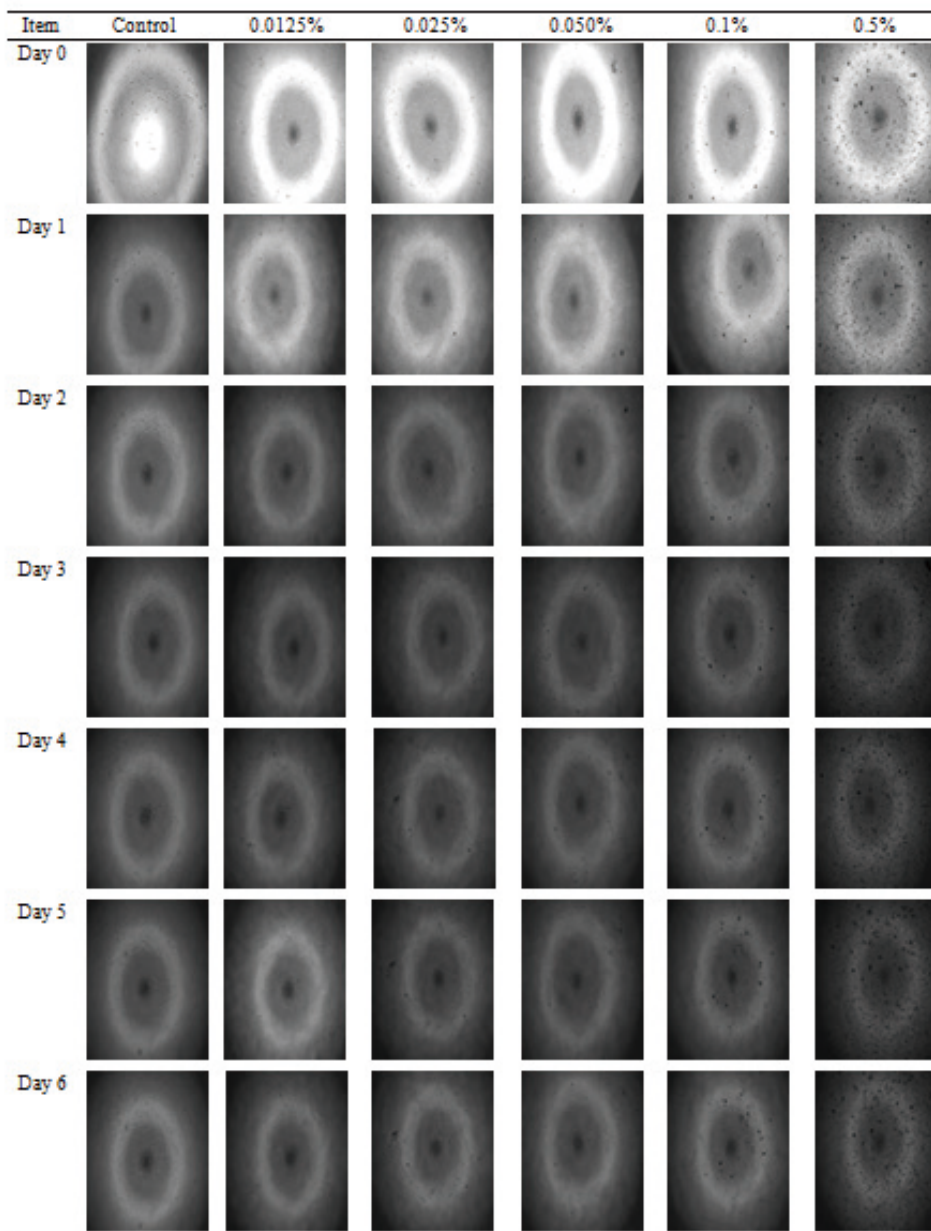


Fig. 4. Morphological comparison between Hanwoo backfat cell. Hanwoo backfat cells were cultured and observed by bright microscopy ($\times 100$).

Discussion

AdN extract (0.125 - 0.250 mg·disc⁻¹) exhibited higher antibacterial effect than the other treatments. However, no antibacterial effect against *Salmonella* was detected using 5.0 mg·disc⁻¹ of AdN extract. In a similar study, a methanol extract of mulberry resulted in a low antibacterial effect against *Bacillus cereus* and *Staphylococcus aureus* at concentrations of 10,000 µg·disc⁻¹. However, the antibacterial effect of the mulberry extracts obtained by methanol and water was not measured at concentrations ranging between 1,000 and 10,000 µg·disc⁻¹ (Kim and Kang, 2012). Antibacterial effects observed in our study might be attributed to flavonoid compounds. Extracts of various medicinal plants containing flavonoids and phenols have been reported to possess antibacterial effect (Rahman et al., 2007; Ayaz et al., 2008). Verbascoside contained in the AdN exhibits various physiological activities (Schlesier et al., 2002). Verbascoside showed a significant antibacterial effect, especially against Gram-positive bacteria (Avila et al., 1999; Nazemiyeh et al., 2008). Despite the availability of antibacterial tests for plant extracts known to contain physiologically active substances, no antibacterial activity was established compared with the physiological activity.

Xanthine oxidase, a non-specific enzyme associated with purine metabolism, generates uric acid from xanthine or hypoxanthine via oxidation (Duke et al., 1973). Xanthine oxidase generates hydroxyl radical (OH[·]), a type of reactive oxygen species (ROS), from hypoxanthine as an oxidizing substrate (Warner and Wispé, 1992). The large amounts of ROS are known to cause skin pigmentation or aging (Chin et al., 2011). The findings suggest that the active oxygen scavenging activity of AdN was comparable to that of allopurinol, which is widely known for its antioxidant effect.

Fibroblasts in the dermal layer contribute to the expression of collagen and elastin. The number and function of fibroblasts in the dermal layer are decreased with age and the decreased moisture content in skin cells reduces the synthesis of proteins such as collagen, elastin, and changes in the stratum corneum (Jeon and Yi, 2014). In a previous study, HFEA (ethyl acetate fraction of *H. fusiformis*) was effective as an anti-aging compound when used on skin due to its antioxidant activity mediated via protection against natural aging or photoaging, and increasing the amount of procollagen type I (Cui et al., 2019). In our experiment, morphological changes and cell viability in the MTS assay following the addition of AdN extract showed no difference; however, the control group showed significantly higher collagen synthesis than the other groups. Further studies are needed to investigate the decrease in collagen synthesis. Certain compounds derived from plants such as polyphenols are prescribed to combat obesity by suppressing adipocyte differentiation and regulating lipid metabolism *in vitro* (Rayalam et al., 2008; Gourineni et al., 2012; Lu et al., 2012). For example, caffeine, a xanthine alkaloid, reduces the accumulation of lipids in adipocytes and enhances lipolysis by inhibiting phosphodiesterase activity (Herman and Herman, 2013). Similarly, AdN was shown to reduce the viability of adipocytes in the MTS assay in our experiment, suggesting that AdN extracts are useful in prevention or treatment of obesity.

Conclusion

In our experiment, *Abeliophyllum distichum* Nakai extract was verified the antibacterial activity and antioxidant. 0.125 mg·mL⁻¹ of AdN extract showed the highest active oxygen scavenging activity and antibacterial activity. So, *Abeliophyllum distichum* Nakai seems to be worth using as a functional cosmetic material for skin aging and wrinkle improvement.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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