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Antioxidant, antimicrobial, tyrosinase inhibitory, alcohol dehydrogenase, and acetaldehyde dehydrogenase activities of roasted *Dendropanax morbifera* Lev. leaves extracted with different ethanol concentrations

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ABSTRACT

Introduction: *Dendropanax morbifera* Lev., an indigenous Korean plant, has a long history of use as a remedy for various maladies such as migraine headache and inflammation. The aim of this study was to evaluate the antioxidant, antimicrobial, and biological (tyrosinase inhibitory, alcohol dehydrogenase [ADH], and acetaldehyde dehydrogenase [ALDH]) activities of roasted *D. morbifera* Lev. leaves extracted with different concentrations of ethanol. **Methods:** Ethanol extracts (0, 20, 40, 60, 80, and 100%) of roasted *D. morbifera* Lev. leaves were examined through 2,2-azino-bis-(3-ethylbenzothiazothiazoline-6-sulfornic acid) and 1,1-diphenyl-1-picrylhydrazyl radical scavenging, total phenol and flavonoid content, reducing power, tyrosinase inhibitory activity, ADH and ALDH activity, and minimum inhibitory concentration assays. **Results and Conclusion:** In most assays, significant activity was observed in the 100% ethanol extract, and the degree of activity decreased with the ethanol concentration. However, the results for the tyrosinase inhibitory activity were different. The 80% and 60% ethanol extracts exhibited the highest *L*-tyrosine and *L*-3,4-dihydroxyphenylalanine activities, respectively. The result suggests that the roasting causes the regeneration and degeneration of phenolic compounds, and possibly other active compounds, contributing to higher activity.

Keywords: *Dendropanax morbifera* Lev., roasting, solvent ethanol concentrations, various activities

INTRODUCTION

Dendropanax morbifera Lev. is a pharmaceutical plant endemic to and traditionally used in southwestern South Korea. Its gold sap is very valuable as a varnish, and its roots, leaves, seeds, and stems have been used to treat for various illnesses including infections and dermal diseases.[1] Chlorogenic acid, rosmarinic acid, and rutin have been identified as intrinsic compounds in the leaves,[2] as well as dendropanoxide, α-amyrin, β-amyrin, β-sitosterol, and α-glutinol.[3] Although some active and non-active compounds have been identified, *D. morbifera* Lev. leaves may contain still-undefined compounds. Various functional studies on *D. morbifera* Lev. have been conducted such as assaying antimicrobial activity by the disc diffusion method measured by the minimum inhibitory concentration. This study demonstrated that an ethanol extract of *D. morbifera* Lev. leaves strongly inhibited *Bacillus cereus* (2.5 ± 0.0 mg/ml), *Staphylococcus aureus* (3.0 ± 0.0 mg/ml), *S. aureus* subsp. *aureus* (4.0 ± 0.0 mg/ml), and *Streptococcus mutans* (5.0 ± 0.0 mg/ml).[4] Kim *et al*.[5] reported that *D. morbifera* Lev. leaf ethanol extract and chloroform fraction notably reduced cisplatin-induced nephrotoxicity in NRK-52E cells and rats, demonstrating a kidney-protective effect *in vitro* and *in vivo*. Moreover, lipopolysaccharide-induced NO and prostaglandin E2 production were greatly inhibited by *D. morbifera* Lev.

methanol extracts made with green and senescence leaves.[6] However, although these studies have indicated a wide range of potential activities using various extraction solvents, the optimal solvent concentration for maximal activity remains unknown. We have previously reported on the biological activities of *D. morbifera* Lev. leaves extracted with different ethanol concentrations and with distilled water.[7] However, some studies have reported increased activity after roasting,[8,9] and no previous investigations have studied the biological activities of roasted *D. morbifera* Lev. Therefore, in this study, the activity of roasted *D. morbifera* Lev. leaves extracted with different ethanol concentrations (0, 20, 40, 60, 80, and 100%) was investigated using various assays.

MATERIALS AND METHODS

Chemical

Trichloroacetic acid, 1,1-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, potassium persulfate, ferric chloride, sodium carbonate, and butylated hydroxytoluene (BHT) were purchased from Wako Pure Chemical Industries Ltd. (Japan). Quercetin, gallic acid, alcohol dehydrogenase (ADH), β-nicotinamide adenine dinucleotide hydrate (NAD), acetaldehyde, *L*-tyrosine, acetaldehyde dehydrogenase (ALDH), and arbutin were obtained from Sigma Chemical Co. (USA). *l*-3,4-dihydroxyphenylalanine (*L*-DOPA) was purchased from Alfa Aesar (USA). 2,2-azino-bis-(3-ethylbenzothiazothiazoline-6-sulfonic acid) (ABTS) was obtained from Amresco LLC (USA). Other reagents were obtained from Daejung Chemical and Metal Co., Ltd (Republic of Korea). All reagents were of analytical grade or better.

Plant Materials, Extract Preparation, and Extraction Yield

Roasted *D. morbifera* Lev. leaves were obtained from Jeju Resource Plant Laboratory in July 2015. They were stored at room temperature for 2 days to eliminate any dampness and then powdered in an electrical blender to a 1 mm particle size. For extraction preparation, 5 g of finely pulverized powder was extracted 3 times with either distilled water or 20, 40, 60, 80, and 100% ethanol at approximately 25°C for 90 min. An ultrasonic bath (Power sonic 520, Hwashin Co., Korea) was employed during extraction to enhance the yield. The extracts were filtered to remove impurities using Whatman No. 2 filtering paper (Whatman International Limited, Kent, England) and concentrated in a rotary vacuum evaporator (Hei-VAP Precision, Heidolph, Germany; at 280 rpm) to obtain a solid dried sample. The sample was weighed, and the extraction yield was calculated as a percentage of the concentrated sample weight (g) of the original dry weight (g). The obtained samples were stored at −20°C until further use.

ABTS Radical Scavenging Capability Assay

The ABTS radical scavenging capability was evaluated according to the method of Ra *et al*.[10] Before the study, 7 mM ABTS and 2.45 mM potassium persulfate were stored for 16 h in the dark to generate ABTS radical. After 16 h, the initial absorbance was measured using UV spectrophotometer at 750 nm (UV-1800, Shimadzu Co., Japan) and adjusted to 1.2–1.3 with sodium phosphate buffer between 1.2 and 1.3. Then, the samples were mixed with ABTS solution and allowed to react for 20 min at room temperature. The absorbance was read on a microplate reader (iMark, Bio-Rad Laboratories, Inc., USA). The ABTS radical scavenging activity was calculated using the following equation:

$$\% \text{ Inhibition} = (1-\text{As/Ac})\times 100$$

Where, As indicates the actual absorbance of the sample and Ac indicates the absorbance of the control without sample solutions. After determining the percentage inhibition, this value was expressed as RC_{50}, which is the amount of sample solution required to scavenge 50% of the free radicals. BHT was used as a reference standard.

DPPH Radical Scavenging Capability Assay

The DPPH radical scavenging capability was adopted according to the procedure of Ra *et al*.[10] To every row except for the second row in a 96-well microtiter plate, 100 μl of methanol and 200 μl diluted sample solutions were added. The sample solutions were serially diluted 7 times subsequent rows. After diluting, 0.15 mM DPPH solution was added to each cell. Then, the reaction solutions were incubated in the dark for 30 min, and the absorbance was determined at 490 nm using a microplate reader. BHT and α-tocopherol were used as reference compounds. The DPPH radical scavenging capability was determined using the same equation for the ABTS and was also expressed as the RC_{50}.

Measurement of Total Phenol and Flavonoid Content

The total content of phenol compounds was estimated according to the method of Nakamura *et al*.[11] First, the sample solutions, 500 μl distilled water and 50 μl Folin–Ciocalteu agent, were mixed and incubated for 5 min at room temperature. Then, sodium carbonate was added to the reaction mixture, which was incubated for 20 min before the addition of 1 ml distilled water. After vigorous vortexing, the absorbance was read at 725 nm using a UV spectrophotometer. The phenol content was calculated using a calibration curve and expressed as mg gallic acid equivalent (GAE)/g dried *D. morbifera* Lev. leaves.

The aluminum chloride colorimetric method described by Nakamura *et al*.[7] was used to measure the total flavonoid content. Diluted sample solution was added to each test tube with 100 μl 10% aluminum nitrate. Then, 1 M potassium acetate was mixed with 80% methanol to a total volume of 5 ml. The solution was incubated for 40 min at ambient temperature, and the absorbance was measured at 415 nm using a UV spectrophotometer. A quercetin calibration curve was plotted to determine the total flavonoid content. The value was expressed as mg quercetin equivalents (QEs)/g dried *D. morbifera* Lev. leaves.

Determination of Reducing Ability Assay

The reducing power assay analyzed according to the method of Nakamura *et al*.[11] The sample solutions were

appropriately diluted with distilled water and mixed with 500 μl of 200 mM sodium phosphate buffer (pH = 6.6). Then, 1% potassium ferricyanide was added to the test tube, which was vigorously shaken and incubated for 20 min at 50°C. Trichloroacetic acid (2.5 ml) was added to terminate the reaction. Finally, a 500 μl aliquot of the mixture was transferred to a microtube and mixed with 0.1% ferric chloride. The absorbance was determined at 700 nm using a UV spectrophotometer. High absorbance indicated high reducing ability.

Determination of Tyrosinase Inhibitory Ability Assay

The tyrosinase inhibitory ability assay was performed according to the method of Ra *et al*.[10] using two substrates, *L*-tyrosine and *L*-DOPA. Phosphate buffer (0.1 M, pH = 6.8) was added to dilute the sample solutions to suitable concentrations and mixed with 1.66 mM *l*-tyrosine (*l*-DOPA). The solutions were mixed with 40 μl tyrosinase (125 units/ml) and 80 μl potassium phosphate buffer and then incubated 37°C for 20 min. Finally, the absorbance was measured using a microplate reader at 495 nm. Arbutin was used as a positive control. The tyrosinase inhibitory activity was calculated by the following formula:

$$\% \text{ Inhibitory activity} = (1 - (A_S/Ab)/Ac) \times 100$$

Where, As is the absorbance of the sample solution and Ac indicates the absorbance of a control containing phosphate buffer instead of sample solutions or arbutin; the blank (Ab) was prepared similarly but without substrates.

ADH and ALDH Activity Assay

The ADH activity of roasted *D. morbifera* Lev. leaves was evaluated using the technique of Nakamura *et al*.[11] First, the sample solutions were mixed with ethanol and NAD solution and shaken vigorously. Glycine-NaOH buffer (pH = 8.8) was added and allowed to react for 10 min at 25°C. ADH solution (10 units/ml) was added to the reaction mixture, and the absorbance was measured at 340 nm using a UV spectrophotometer. The control contained the reactants without sample solution, and its value is shown as 100% in the graph.

The ALDH activity was determined using the method described by Nakamura *et al*.[11] A reaction mixture containing 50 mM sodium phosphate buffer, acetaldehyde, and 0.5 mM NAD solutions mixed with 0.1 mM ALDH solution (1 unit/ml) and incubated for 10 min at 37°C. After the incubation, the absorbance was determined at 370 nm using a UV spectrophotometer. The control contained the reactants without sample solution, and its value is shown as 100% in the graph.

Minimum Inhibitory Concentration Assay

As an antimicrobial assay, the serial 2-fold dilution method of Nakamura *et al*.[11] was employed to determine the inhibition of microbial multiplication. All species used in this assay were purchased from the Korean Collection for Type Culture (KCTC). Three Gram-positive bacteria (*B. cereus* [KCTC 1012], *Staphylococcus epidermidis* [KCTC 1917], and *S. aureus* subsp. *aureus* [KCTC 1927]), three Gram-negative bacteria (*Pseudomonas aeruginosa* [KCTC 1750], *Escherichia coli* [KCTC 1924], and *Klebsiella pneumonia* subsp. *pneumonia* [KCTC 2208]), and two fungi (*Saccharomyces cerevisiae* [KCTC 7913] and *Pichia jadinii* [KCTC 7293]) were used to determine the antimicrobial activity of *D. morbifera* Lev. leaves. Bacteria were incubated at 30°C or 37°C in nutrient agar, and fungi were incubated at 25°C or 26°C in yeast mold agar. Growth inhibition was evaluated by the degree of murkiness in each cell through naked eye observations over 20 h with two replicates.

Determination of Correlation and Data Analysis

All data are expressed as the mean ± standard deviation of representative duplicate experiments, and statistical significance was determined by one-way analysis of variance, with $P < 0.05$ considered statistically significant. When $P < 0.05$, Duncan's multiple range test was performed to determine the statistical significance between mean values. To identify correlation among the assays, Pearson's correlation coefficient was used (all analyses were performed using SAS Statistical Package for the Social Sciences, Ver. 20.0 [SAS Institute Inc., Cary, NC, USA]).

RESULTS AND DISCUSSION

Yield of Roasted *D. morbifera* Lev. Leaves Extracted with Different Ethanol Concentrations

The extraction yield increased up to the 40% ethanol concentration and then dramatically decreased at higher concentrations [Table 1] consistent with previous related study.[12] As Chen *et al*.[13] suggested, mixing ethanol and water broadens the extraction range, and the water in the solvent might lead to the swelling of some plant tissues, resulting in an increased surface area contacting the solvent. This hydroethanolic solution could extract more constituents compared to pure ethanol or distilled water extract. This clearly indicates that water content in the ethanol increased the extraction yield and probably resulted in the swelling of the roasted *D. morbifera* Lev. leaf tissue, making it more extractable. In contrast, pure ethanol extraction demonstrated a much lower extraction yield compared to distilled water and the hydroethanolic extracts. The same phenomenon was observed in our previous study using different ethanol concentrations to produce unroasted *D. morbifera* Lev. leaf extracts.[7] These results indicate that 40% ethanol is the optimal concentration to retrieve the most compounds, including possibly active compounds, from roasted *D. morbifera* Lev. leaves.

ABTS Radical Scavenging Capability

Contrary to the extraction yield results, scavenging capability was directly dependent on the ethanol concentration, i.e., high ethanol concentrations showed higher ABTS radical scavenging capabilities and vice versa [Table 1]. The radical scavenging capabilities were as follows: 72.4 ± 3.5 μg/ml for distilled water, 48.00 ± 0.78 μg/ml for 20% ethanol extract,

Table 1: Results of yield, ABTS and DPPH radical scavenging, and total phenol and flavonoid content assays for roasted *Dendropanax morbifera* Lev. leaf extracts in distilled water and different concentrations of ethanol

Extract concentrations†	Yield (%)	ABTS and DPPH radical scavenging capability (μg/ml)		Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)
		RC_{50} ††	RC_{50} †††		
H2O	24.80±0.21^{b}††††	72.4±3.5^{g}	43.17±0.49^{g}	1484.7±12.1^{e}	189.5±7.5^{d}
20E	25.65±0.90^{ab}	48.00±0.78^{f}	34.49±0.44^{e}	1536.1±4.0^{d}	204.0±1.9^{cd}
40E	26.69±0.67^{a}	35.8±1.5^{e}	21.87±0.63^{d}	1630.5±16.1^{c}	206.7±5.6^{cd}
60E	10.22±0.35^{c}	22.7±1.4^{d}	20.5±1.1^{d}	1690.5±28.3^{b}	222.5±16.8^{c}
80E	9.60±0.33^{c}	15.3±1.7^{c}	11.01±0.13^{c}	1782.0±20.2^{a}	292.6±15.0^{b}
100E	8.95±0.10^{c}	10.08±0.26^{b}	9.30±0.72^{b}	1824.9±24.3^{a}	365.4±5.6^{a}
Toco		4.61±0.06^{a}	4.06±0.01^{a}		
BHT			40.9±1.0^{f}		

†H_2O: Distilled water, 20E: 20% ethanol extract, 40E: 40% ethanol extract, 60E: 60% ethanol extract, 80E: 80% ethanol extract, 100E: 100% ethanol extract, Toco: α-tocopherol, ††The concentration required to reduce 50% of ABTS radical, †††The concentration required to reduce 50% of DPPH radical, ††††Each value represents the mean±SD. Means within column labeled with the same letters are not significantly different ($P<0.05$). DPPH: 1,1-diphenyl-1-picrylhydrazyl, ABTS: 2,2-azino-bis-(3-ethylbenzothiazothiazoline-6-sulfonic acid), GAE: Gallic acid equivalent, QEs: Quercetin equivalents, BHT: Butylated hydroxytoluene

35.8 ± 1.5 μg/ml for 40% ethanol extract, 22.7 ± 1.4 μg/ml for 60% ethanol extract, 15.3 ± 1.7 μg/ml for 80% ethanol extract, and 10.08 ± 0.26 μg/ml for 100% ethanol extract, respectively, with 100% ethanol extraction generating the highest ABTS radical scavenging capability. Another study reported that a distilled water extraction of black onion scavenged ABTS radicals much more strongly compared to a crude ethanol extraction.[14] Further, Yoon *et al*.[15] performed ABTS radical scavenging assay to evaluate the antioxidant potential of *Aconiti koreani Rhizoma* with different extraction solvent ratios (0, 20, 40, 60, 80, and 100% ethanol). The scavenging activity was concentration dependent up to 60% ethanol, but then decreased dramatically, consistent with the results of our study. This discrepancy may be due to different intrinsic compounds attracting solvents in different manners. It is believed that compounds active against ABTS radicals would be moderately polar, and such compounds may be present in roasted *D. morbifera* Lev. leaves.

DPPH Radical Scavenging Capability

Table 1 summarizes that all extracts demonstrated DPPH radical scavenging capability ranging from 9.30 ± 0.72 to 43.17 ± 0.49 μg/ml. Similar to the ABTS radical scavenging capability assay, the DPPH radical was also most highly scavenged by the 100% ethanol extract, whereas the lowest activity was exhibited by the distilled water extract. The scavenging capability became stronger as concentration increased, and extracts with lower ethanol concentrations (below 50%) also exhibited high DPPH radical scavenging capability (34.49 ± 0.44 and 21.87 ± 0.63 μg/ml for the 20 and 40% ethanol concentrations, respectively). We also compared α-tocopherol and BHT with the sample solutions, and while none of extracts performed better than α-tocopherol (4.06 ± 0.01 μg/ml), the performances of the 100 and 80% ethanol extracts (11.01 ± 0.13 μg/ml and 9.30 ± 0.72 μg/ml, respectively) were only slightly lower. Furthermore, the radical capability of all extracts except for the distilled water extract exceeded that of BHT (40.9 ± 1.0 μg/ml). Ju *et al*.[16] reported that high ethanol concentrations (90–100%) exhibit lower DPPH radical scavenging activity, inconsistent with our result. Lou *et al*.[17] extracted *Citrus mitis* Blanco using boiling water (80, 90, and 100°C) and found that the constituents were broken down by the heat, but other compounds were actually induced to a certain extent. One possible explanation might be that some of compounds decomposed during the roasting process, while other compounds have undergone property change or been induced to some extent. High-concentration ethanol extraction (80 and 100%) resulted in strong DPPH radical scavenging capability, much higher than that of propolis, a traditional medicine dating back a thousand years and containing various phenolic acid and flavonoid compounds, when extracted by distilled water and various ethanol concentrations (25, 50, 75, 95, and 100%).[18]

Total Phenol and Flavonoid Content

The total phenol and flavonoid contents showed consistent trends to the radical scavenging activities described above, i.e., 100% ethanol extract exhibited the highest phenol and flavonoid contents, whereas distilled water had the lowest. The total phenol contents in the sample solutions are listed in Table 1. Values ranged from 1000 to 2000 mg GAE/g. The highest phenol content was observed after 100% ethanol extraction (1824.9 ± 24.3 mg GAE/g), followed by 80% (1782.0 ± 20.2 mg GAE/g), 60% (1690.5 ± 28.3 mg GAE/g), 40% (1630.5 ± 16.2 mg GAE/g), and 20% ethanol extract (1536.1 ± 4.0 mg GAE/g), and distilled water extract (1484.7 ± 12.1 mg GAE/g). A summary of total flavonoid content in *D. morbifera* Lev. leaves extracted with different ethanol concentrations is also shown in Table 1. Roasted *D. morbifera* Lev. leaf extracted with 100% ethanol exhibited the highest content with 365.4 ± 5.6 mg QE/g. In contrast, the distilled water extract exhibited the lowest flavonoid content with 189.5 ± 7.5 mg QE/g, just over half that observed with 100% ethanol. The amount of flavonoid increased as the concentration increased. Overall, higher ethanol concentrations extracted higher amounts of phenols and flavonoids in roasted *D. morbifera* Lev. leaves. The same trend was observed by Sun *et al*.[18] However, another study

examined the highest total phenol content in black onion extracted with distilled water, ethanol, and methanol.[14] The extractable phenolic compounds and their degrees of accumulation vary depending on the plant and its parts. For roasted *D. morbifera* Lev. leaves, ethanolic solutions retrieved markedly more phenolic compounds than distilled water, indicating that non-water-soluble or moderately soluble phenolic compounds probably comprise most of the total phenol and flavonoid content. Chlorogenic acid, rutin, and rosemarinic acid were identified after methanol extraction of *D. morbifera* Lev. leaves.[2] Notably, 100% ethanol exhibited the lowest extraction yield, but the phenol and flavonoid contents were higher than in the other extracts. This suggested that phenolic compounds may predominate in the 100% ethanol extract, whereas in distilled water, non-phenolic compounds may exist to a greater extent. According to Yang *et al.*,[19] phenolic compounds are frequently involved with overall biological activity, showing strong positive or negative correlation. The assays in this study revealed that extracts with the highest total phenolic content exhibited both the highest activity and lowest extraction yield. This suggests that phenolic compounds are virtually solely responsible for the biological activity of *D. morbifera* Lev. leaves.

Reducing Ability Assay

As shown in Figure 1, the reducing power increased with increasing ethanol concentration, and 100% ethanol extract exhibited the strongest reducing ability at all concentrations (100, 200, and 300 μg/ml), whereas distilled water extract had the weakest reducing ability. Furthermore, as more sample solution was dispensed, the activity got stronger, whereas Akkanii *et al.*[20] reported that the reducing power of *Kigelia africana* distilled water extract actually decreased from 10 to 250 μg/ml but improved from 250 to 1000 μg/ml. However, another study reported increased reducing power with increased dose.[21] It seems that plants mostly accumulate weak or non-active compounds during their growing periods, which exhibit in sufficient reducing power to show dose-dependent behavior at lower concentrations. Baba *et al.*[22] investigated barley flour treated by microwave roasting and found that roasting the flour-induced significant reducing power compared to the native flour. During the roasting process, the substances produced by the Maillard reaction would increase the reducing power. In our study, such substances function as effective reductones, with 100% ethanol exhibited the highest reducing power among the other extracts.

Tyrosinase Inhibitory Ability

Unlike other assays in the present study, 80% ethanol extract was the most effective in *L*-tyrosine inhibition, whereas 60% ethanol extract exhibited the highest *L*-DOPA inhibitory activity [Figure 2]. The inhibition rates of the extracts in this study were stronger than those reported by Khanom *et al.*[23] (with the exception of distilled water, and excluding *Glycyrrhiza glabra*, which showed 97.9% inhibition), where the tyrosinase inhibitory activity using *l*-tyrosine as the substrate was 8.3–97.9% for 16 Bangladesh indigenous medicinal plants. According to Maisuthisakul and Gordon,[24] the tyrosinase inhibitory activity is highly consistent with the phenol content, and in this study, the mango seed kernel by-product (sun-dried and oven-dried) that possessed the highest phenolic content had the highest tyrosinase inhibitory activity. In contrast, the results obtained in the present study rather supported those of Shukla *et al.*,[25] who observed a weak correlation between the total phenol content and the tyrosinase inhibitory activity. Relationships between the phenol content and tyrosinase inhibition may depend on –OH groups in the phenolic compounds, which can suppress enzymatic activity or cause conformational changes. These results suggest that tyrosinase inhibition may involve phenolic compounds, which may act as tyrosinase or cofactors or substrates.[26] Further, hydroethanolic solutions demonstrated higher activities compared to distilled water, indicating that most tyrosinase inhibitors are water-insoluble compounds.

ADH and ALDH Activity Assay

ADH and ALDH activity increased with the dose and ethanol concentration [Figure 3]. Except for the 80% and 100% ethanol extract, the extracts in this study did not exhibit significant activity. In particular, the distilled water and 20% ethanol extract demonstrated lower ADH activity. This indicates that few extracted active compounds dissolved in water or that the water-soluble compounds present were weak. Minerals such as Ca^{2+} and Zn^{2+} and amino acids such as aspartic acid also facilitate ADH and ALDH activity. These moderately water-soluble compounds reactivate and enhance enzyme activities as previously reported.[27,28] Further,

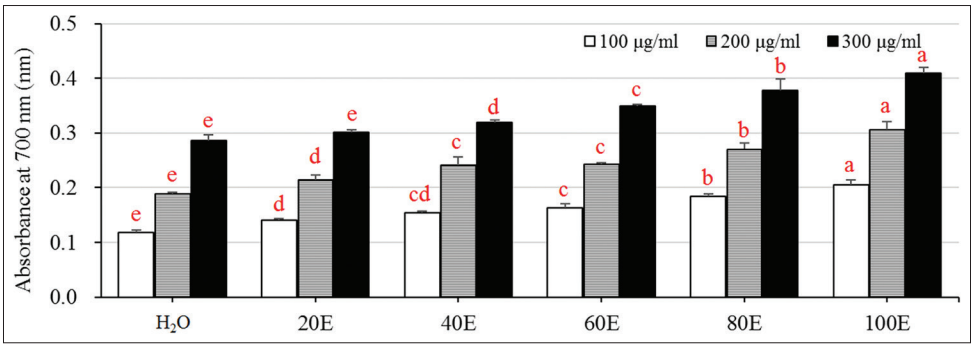

Figure 1: Reducing ability of roasted *Dendropanax morbifera* Lev. leaves with extracted different ethanol concentrations. H_2O: Distilled water extract, 20E: 20% ethanol extract, 40E: 40% ethanol extract, 60E: 60% ethanol extract, 80E: 80% ethanol extract, 100E: 100% ethanol extract. Values with different letters are significantly different as calculated using a paired Duncan's multiple range test at $P < 0.05$

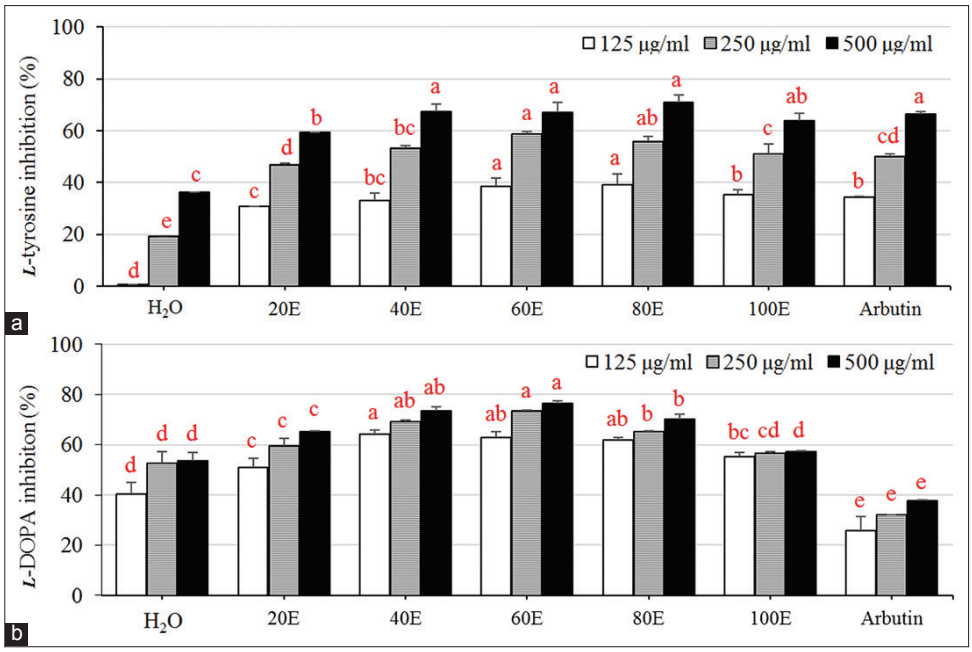

Figure 2: Tyrosinase inhibitory activity (*L*-tyrosinase [a] and *L*-3,4-dihydroxyphenylalanine [b]) of roasted *Dendropanax morbifera* Lev. leaf extracts. H_2O: Distilled water extract, 20E: 20% ethanol extract, 40E: 40% ethanol extract, 60E: 60% ethanol extract, 80E: 80% ethanol extract, 100E: 100% ethanol extract. Values with different letters are significantly different as calculated using paired Duncan's multiple range test at $P < 0.05$

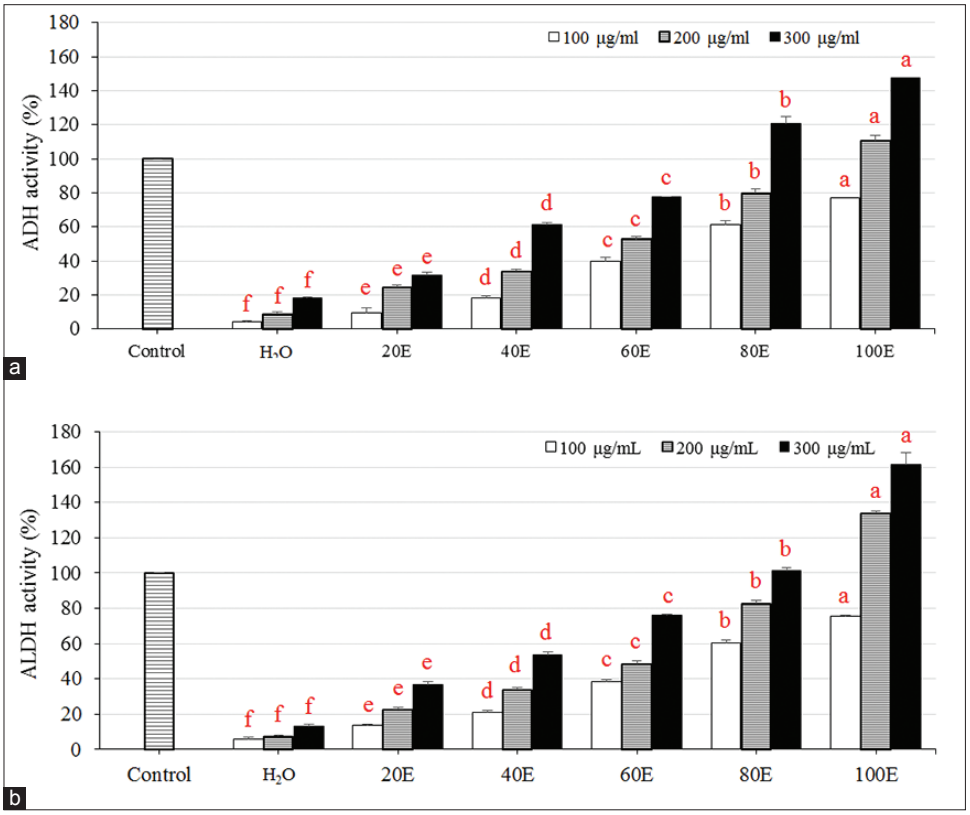

Figure 3: Alcohol dehydrogenase (a) and acetaldehyde dehydrogenase (b) activities of roasted *Dendropanax morbifera* Lev. leaf extracts. H_2O: Distilled water extract, 20E: 20% ethanol extract, 40E: 40% ethanol extract, 60E: 60% ethanol extract, 80E: 80% ethanol extract, 100E: 100% ethanol extract. Values with different letters are significantly different as calculated using paired Duncan's multiple range test at $P < 0.05$

according to Jung and Kang,[29] boiling water extracts of three vegetables (*Glycine max*, *Oenanthe javanica*, and *Raphanus sativus* var. *hortensis for. acanthiformis*) exhibited relatively lower activity compared to distilled water and methanol

extract. They hypothesized that the boiling process destroys or denatures the apoenzyme, which lacks cofactor activity and acts to transport chemical groups to other enzymes. In the present study, the *D. morbifera* Lev. leaves were also heat-treated, although through a different process. Therefore, some of the active compounds effective on ADH or ALDH may have been denatured during roasting. The ADH and ALDH activities of unroasted *D. morbifera* Lev. leaves were not significant.[7]

Antimicrobial Activity with Minimum Inhibitory Concentration

The antimicrobial activity of roasted *D. morbifera* Lev. leaves was investigated, and inhibition of specific microbial strains was observed in the 40, 60, 80, and 100% ethanol extracts. The distilled water and 20% ethanol extract did not show significant microbial inhibition, similar to the results of Kim *et al*.[30] This suggests that the compounds inhibiting bacterial growth are mostly water insoluble. This makes sense since most identified active compounds, such as chlorogenic acid or β-sitosterol, were isolated using methanolic solvent.[2,3] The strong antimicrobial activities of these compounds have been demonstrated in previous studies.[31,32] Marked inhibition was observed at higher ethanol concentrations (60–100%) in our study, and the above-mentioned compounds might dissolve in ethanolic extracts, blocking bacterial cell membrane permeability changes. Research has indicated that roasting decreases antimicrobial ability,[33] but in this study, the roasting process slightly increased the antimicrobial potency of *D. morbifera* Lev. leaves compared to previous research.[7] Bisignano *et al*.[34] found that the roasting process induces the formation of some active compounds including chlorogenic acid in *Pistacia vera* L. or induces structural changes in them. Thus, it is possible that some active antimicrobial compounds were regenerated or enhanced in the roasted *D. morbifera* Lev. leaves, leading to higher antimicrobial activity [Table 2].

Correlation Analysis among Antioxidant and Biological Activities

As shown in Table 3, total phenol content was strongly correlated with the reducing power (0.963, $P < 0.001$), DPPH radical scavenging (−0.976, $P < 0.001$), ABTS radical scavenging activity (−0.955, $P < 0.001$), ADH activity (0.970, $P < 0.001$), and ALDH activity (0.970, $P < 0.001$). Total flavonoid content was also highly consistent with the reducing power (0.933, $P < 0.001$), DPPH radical scavenging activity (−0.811, $P < 0.01$), ABTS radical scavenging activity (−0.782, $P < 0.01$), ADH activity (0.945, $P < 0.001$), and ALDH activity (0.952, $P < 0.001$). Although which phenol compounds affect specific activities remains unknown, this indicated that strong antioxidant, ADH, and ALDH activities mainly relied on the overall phenolic content. Compared with unroasted leaves,[7] the correlation value was significantly improved, suggesting that roasting improve the activity of *Dendropanax morbifera* Lev. leaf extracts. However, total flavonoid content was poorly correlated with *l*-tyrosine (0.474, $P > 0.05$) and *l*-DOPA inhibitory activities (0.262, $P > 0.05$). Between total phenol content and *l*-tyrosine and *l*-DOPA inhibitory activity demonstrated relatively weak correlations as well. From a statistical point of view, this suggests that non-phenolic compounds may be responsible for *L*-tyrosine and *L*-DOPA inhibitory activity.

CONCLUSION

We have investigated the activity of roasted *D. morbifera* Lev. leaves extracted with different ethanol concentrations. The 100% ethanol extract exhibited the highest activity in most assays, and generally, extract activity increased with increased ethanol concentration. However, tyrosinase inhibitory activity showed a different pattern, with the 80 and 60% ethanol extracts showing the highest inhibitory activity against *L*-tyrosine and *L*-DOPA, respectively. Since the 100% ethanol extract had the highest total phenol and flavonoid content, the compounds effective at tyrosinase inhibition

Table 2: Minimum inhibitory concentration of *Dendropanax morbifera* Lev. leaf extracts against various microbes

Minimum inhibitory concentration (μg/ml)							
Extract†/Bacterial strain††	**H_2O**	**20E**	**40E**	**60E**	**80E**	**100E**	**Tetracycline**
B. c	-†††	-	1000	500	250	62.5	7.8
S. e	-	-	-	-	-	-	7.8
S. a	-	-	1000	1000	500	125	7.8
P. a	-	-	-	-	-	-	15.6
E. c	-	-	1000	250	250	125	7.8
K. p	-	-	-	-	-	-	7.8
P. v	-	-	-	-	-	-	7.8
S. c	-	-	-	-	-	-	
P. j	-	-	1000	250	125	125	
C. a	-	-	-	-	-	-	

†H_2O: Distilled water extract, 20E: 20% ethanolic extract, 40E: 40% ethanolic extract, 60E: 60% ethanolic extract, 80E: 80% ethanolic extract, 100E: 100% ethanolic extract, ††B.c: *B. cereus* (KCTC 1012), S.e: *Staphylococcus epidermidis* (KCTC 1917), S.a: *Staphylococcus aureus* subsp. *aureus* (KCTC 1927), P.a: *Pseudomonas aeruginosa* (KCTC 1750). E.c: *Escherichia coli* (KCTC 1924). K.p: *Klebsiella pneumoniae* subsp. *pneumoniae* (KCTC 2208). P.v: *Proteus vulgaris* (KCTC 2433), S.c: *Saccharomyces cerevisiae* (KCTC 7913), P.j: *Pichia jadinii* (KCTC 7293), C.a: *Candida albicans* (KCTC 7965), †††>1000 μg/ml, KCTC: Korean Collection for Type Culture

Table 3: Assay correlation analysis for *Dendropanax morbifera* Lev. leaf extracts

Assays†	TP	TF	RP	DPPH	ABTS	TY	DO	ADH	ALDH
TP	1	0.873***††	0.963***	−0.976***	−0.955***	0.741**†††	0.614*††††	0.970***	0.970***
TF		1	0.933***	−0.811**††	−0.782**	0.474	0.262	0.945***	0.952***
RP			1	−0.922***	−0.909***	0.667**	0.484	0.983***	0.982***
DPPH				1	0.978***	−0.821**	−0.746**	−0.919***	−0.923***
ABTS					1	−0.884***	−0.767**	−0.905***	−0.907***
TY						1	0.848***	0.630*	0.641*
DO							1	0.462	0.46
ADH								1	0.998***
ALDH									1

†TP: Total phenol content, TF: Total flavonoid content, RP: Reducing power ability assay, TY: *L*-tyrosine inhibitory activity, DO: *L*-DOPA inhibitory activity, ††***significance $P<0.001$, †††**significance $P<0.01$, ††††*significance $P<0.05$, ADH: Alcohol dehydrogenase, ALDH: Acetaldehyde dehydrogenase

may have been non-phenolic. In addition, roasting may have resulted in regeneration or denaturation of some phenolic and other active compounds, resulting in increases or decreases in radical scavenging, reducing power, antimicrobial, ADH, and ALDH activities as well as the total phenol and flavonoid contents. Our results indicate that roasted *D. morbifera* Lev. leaves possess various remarkable abilities and should be considered in the development of pharmaceutical products. In future research, more details on the structure and function of the active compounds should be obtained to better understand their advantageous properties.

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