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## Original Article

# Impact of different partitioned solvents on chemical composition and bioavailability of *Sasa quelpaertensis* Nakai leaf extract



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## ABSTRACT

The leaves of *Sasa quelpaertensis* Nakai were extracted with 80% ethanol and further partitioned with *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous fractions to evaluate the biological activity through assessment via various *in vitro* assays, including total phenol content; 1,1-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3-ethylbenzothiazothiazoline-6-sulfonic acid (ABTS) radical scavenging; reducing power;  $\alpha$ -glucosidase and tyrosinase inhibitory; and alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activity assays. The highest activity was found in the ethyl acetate fraction for all assays and showed stronger DPPH radical scavenging, reducing power, and tyrosinase inhibitory activity than the positive controls (butylated hydroxytoluene,  $\alpha$ -tocopherol, and arbutin, respectively). When compared to the ethyl acetate fraction, the *n*-butanol fraction had lower rates, but it still demonstrated relatively high activity. The activity of the *n*-hexane fraction was high for DPPH and ABTS radical scavenging activity and contained significant amounts of phenol content, whereas the chloroform fraction possessed the highest reducing power, tyrosinase inhibitory, and ADH and ALDH activity, despite having the lowest phenol content when compared to the other fractions. These findings clearly indicate that *S. quelpaertensis* Nakai leaves can be a good natural source of antioxidants and tyrosinase inhibitors, as well as ADH and ALDH activity inducers, suggesting that may have potential for treating various diseases and improving human health.

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## 1. Introduction

Bamboo grass, also called the genus *Sasa*, is widely distributed and cultivated in Asian countries. The leaves of the genus *Sasa* have been used and consumed for the treatment of hypertension, inflammation, and cancer [1]. Recently, Hayashi et al [2] reported that a hot water fraction and eight isolated polysaccharides of *Sasa veitchii* leaves significantly reduced infection of the influenza A virus in rat lung and bronchoalveolar lavage fluid. In another study, two new compounds (Kurilensin A and B) and several already-known compounds (tricin-4'- $\beta$ -D-glucopyronoside and tricin-5-O- $\beta$ -D-glucosepyronoside) were isolated from *S. kurilensis* leaves, and the DPPH (1,1-diphenyl-1-picrylhydrazyl) radical scavenging activity of the two new compounds was nearly twice times as high (IC<sub>50</sub> of 6.0 and 5.1  $\mu$ M, respectively) as the activity of ascorbic acid (IC<sub>50</sub> of 12  $\mu$ M) [3].

*Sasa quelpaertensis* Nakai also belongs to the genus *Sasa*, and this unique bamboo only inhabits Mt. Halla on Jeju Island, South Korea. Its leaves are desiccated and prepared as tea for the treatment of diabetes and gastritis according to the principles of folk medicine in the local community [4]. As more research about the beneficial functions of *S. quelpaertensis* Nakai leaves has emerged, both previously known and unknown properties have been discussed in increasing detail. For instance, Kim et al [5] conducted a study on how effective the distilled water extract of *S. quelpaertensis* Nakai leaves and *p*-coumaric acid can be in attenuating oleic acid-induced lipid accumulation in human hepatoma cells, or HepG2 cells, resulting in a reduction of lipid accumulation by enhancing AMPK phosphorylation. *S. quelpaertensis* Nakai leaves have also been shown to be effective as an anti-inflammatory agent, reducing the amount of cyclooxygenase-2, tumor necrosis factor- $\alpha$ , and inducible nitric oxide synthase by inhibiting the rapid increase in the number of colon cells and by regulating two specific signaling pathways [6]. Furthermore, it has been reported that the replication of porcine reproductive and respiratory syndrome virus (PRRSV), the virus that causes serious damage in the respiratory system in pigs and reproductive failure in pregnant sows, was suppressed by the distilled water extract of *S. quelpaertensis* Nakai leaves in PAM-KNU cells. A growth kinetics study further supported the findings concerning these benefits by showing that the distilled water extract of *S. quelpaertensis* Nakai leaves significantly slowed down the overall process of virus replication [7]. Nevertheless, *S. quelpaertensis* Nakai is a highly invasive species with strong reproductive ability, and it is therefore a constant annoyance to local people and the diverse environment around Mt. Halla. Consequently, some valuable indigenous plants, such as *Trifolium lupinaster* for. *alpinus* (Nakai) M. Park, are on the verge of extinction [8]. As a result of such threats that the plant poses, practical measures to keep *S. quelpaertensis* Nakai in check while still preserving the species are urgently required on Jeju Island.

Thus, as an attempt to find a way in which to manage the excessive growth effectively by using the plant in the industrial field, the aim of this study is mainly focused on evaluating the biological value of *S. quelpaertensis* Nakai in terms of its antioxidant activity [DPPH, 2,2-azino-bis-3-

ethylbenzothiazothiazoline-6-sulformic acid (ABTS), radical scavenging activity, and reducing power assay], tyrosinase inhibitory activity, and alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activity. Simultaneously, the total phenol content assay was determined in order to analyze the relationship between each assay. To the best of our knowledge, this study represents the first published report on ADH and ALDH activity for *S. quelpaertensis* Nakai leaves. In addition, as described in the study by Kim and Kim [9], the solvent and its polarity is such that researchers have to consider these variables attentively given their relevance to the final extraction yield. As we have previously investigated *S. quelpaertensis* Nakai leaves extracted with different ethanol concentrations to determine the best extraction conditions (data not shown), this study is a continuation of this prior work in order to seek more suitable extraction conditions for the extract (80% ethanol) and its partitioned fractions (*n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous).

## 2. Methods

### 2.1. Plant material

Dried leaves of *S. quelpaertensis* Nakai were purchased in 2015 from the Jeju Plant Resource Lab. The dried leaves were ground into coarse powder to maximize the extraction yield. Pulverized fine power (5 g) was extracted for 20 times more volume with an 80% ethanol solution (v/v) by exposing the dry weight to an ultrasonic bath (Power sonic 520, Hwashin Co., Yeongcheon, Korea) for 90 minutes at approximately 25°C. After 90 minutes, the extracted solution was filtered with Whatman No. 2 filtering paper (Whatman International Ltd., Kent, England, UK) twice to remove insoluble matrices. The extraction process was conducted three times to retrieve more of the active compounds. After the three extractions, the percolated extracts were subjected to concentration by a rotary vacuum evaporator (Hei-VAP Precision 280 rpm; Heddolph, Schwabach, Germany). Then, an 80% ethanol sample was set aside, and the remaining concentrates of 80% ethanol was dissolved by water and then partitioned with *n*-hexane, chloroform, ethyl acetate, *n*-butanol (water-saturated), and distilled water. Each sample was stored in a refrigerator at –20°C until further analysis. The extraction yield was determined by the following formula:

$$\text{Extraction yield (\%)} = (\text{weight of dry soluble solid (g)}/\text{weight sample (g)}) \times 100$$

### 2.2. Determination of total phenol content

The method used by Hyun et al [10] was applied in our study to determine the total amount of phenol content. In brief, a diluted sample solution and 500  $\mu$ L of distilled water were mixed with the Folin–Ciocalteu reagent in a microtube. The test tube was then vortexed for 10 seconds, followed by 5 minutes of incubation at room temperature. After 5 minutes, 300  $\mu$ L of 20% sodium carbonate were added, and the mixture was allowed to stand for 20 minutes. Prior to recording the

absorbance at 725 nm with a UV spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan), 1 mL distilled water was added into the mixture. The experiments were performed in triplicate, and the results were expressed in terms of mg of gallic acid equivalent (GAE) per 1 g *S. quelpaertensis* Nakai leaves (mg GAE/g of *S. quelpaertensis* Nakai leaves).

### 2.3. Analysis of DPPH radical scavenging activity

The DPPH radical scavenging activity was measured according to the same method adopted by Blois [11]. First, to the first row and the third to the end row of a 96-well microtiter plate, 100  $\mu$ L methanol was added. A diluted sample solution (200  $\mu$ L) was placed into the second row and serially diluted to be at lower concentrations toward the end of the row (1.56–200  $\mu$ g/mL). Then, 100  $\mu$ L of 0.15mM DPPH solution was dispensed into all of the cells. After incubation for 30 minutes in the aphotic zone, the absorbance was read with a microplate reader (iMark; Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 490 nm. Finally,  $\alpha$ -tocopherol and butylated hydroxytoluene (BHT) were used as positive controls and DPPH radical scavenging activity was calculated with the following formula:

$$\text{DPPH radical scavenging activity (\%)} = (1 - A_{490} \text{ in sample} / A_{490} \text{ in control}) \times 100$$

The actual value was expressed as  $RC_{50}$ , which is the amount of the sample solution with 50% DPPH radical scavenging after incubation. A lower value indicates higher DPPH radical scavenging activity.

### 2.4. Analysis of ABTS radical scavenging activity

The ABTS radical scavenging activity was determined according to the methods of Re et al [12] with slight modification. First, 7mM ABTS and 2.45mM potassium persulfate were mixed and left for 16 hours in a dark chamber prior to the experiment. After 16 hours, the ABTS solution was diluted with a sodium phosphate buffer to adjust from 1.2 to 1.3 absorbance at 750 nm as determined by a UV spectrophotometer. Each sample solution (40  $\mu$ L) was mixed with ABTS solution and left to react for 20 minutes at room temperature. Then, the absorbance was measured with a microplate reader at 750 nm. As a positive control,  $\alpha$ -tocopherol was used. The ABTS radical scavenging ability was calculated with the following formula:

$$\text{ABTS radical scavenging activity (\%)} = (1 - A_{750} \text{ in sample} / A_{750} \text{ in control}) \times 100$$

After calculating the scavenging rate with the above formula, the value was expressed as  $RC_{50}$ , the required amount for 50% ABTS radical scavenging. The lower the value, the higher the ABTS radical scavenging activity.

### 2.5. Determination of reducing power

The reducing capability of the sample solution was determined spectrophotometrically according to the method

adopted by Hyun et al [10]. First, 100  $\mu$ L of the sample solution for each of the concentrations (100, 200, and 300  $\mu$ g/mL) was mixed with a 0.2M sodium phosphate buffer (pH 6.6) and 500  $\mu$ L of potassium ferricyanide. After 20 minutes of incubation at 50°C, 250  $\mu$ L of 10% trichloroacetic acid was added to the test tube to stop the reaction, and 500  $\mu$ L of the aliquot mixture was transferred into the microtube. Then, 0.1% ferric chloride was mixed with the reactant before absorbance was measured at 700 nm with a UV spectrophotometer. A high optical density meant a stronger reducing ability. Finally,  $\alpha$ -tocopherol was compared with the sample solution.

### 2.6. Inhibition of tyrosinase activity

Tyrosinase inhibitory activity was determined based on how much L-dopaquinone was generated as a by-product during the reaction process by using the method of Huang et al [13] with minor modifications. Sample solutions were diluted with 0.1M potassium phosphate buffer (pH 6.8) and mixed with 40  $\mu$ L of L-tyrosine (1.66mM). Prior to adding 40  $\mu$ L of L-tyrosine solution (125 units/mL), 80  $\mu$ L of 0.1M potassium phosphate buffer was put into the reactants. Then, the solution was incubated for 20 minutes at 37°C in order to read the absorbance at 495 nm with a microplate reader. Instead of L-tyrosine, L-3,4-dihydroxyphenylalanine (L-DOPA) served as the substrate. Arbutin, a whitening agent, was used as a positive control for comparative purposes. The control was prepared without adding the sample solution, and a blank was prepared without L-tyrosine (L-DOPA). Tyrosinase inhibitory activity was calculated as follows:

$$\text{Tyrosinase inhibitory activity (\%)} = [1 - (A_{495} \text{ in sample} / A_{495} \text{ in blank}) / A_{495} \text{ in control}] \times 100$$

### 2.7. ADH and ALDH inducing activity assay

The ADH enhancement ability of *S. quelpaertensis* Nakai leaf extract and its fractions was measured using the technique described by Shim et al [14]. Initially, ethanol, Nicotinamide adenine dinucleotide (NAD) solution, and 100  $\mu$ L of a sample solution were placed into the test tube and vortexed. Then, the glycine–NaOH buffer (pH 8.8) was added to an adjusted level of 1.8 mL, and the mixture was allowed to stand for 10 minutes at 25°C. Finally, ADH solution (10 units/mL) was added to the mixed solutions. The absorbance of each mixed solution was recorded at 340 nm with a UV spectrophotometer.

The ALDH enhancement activity for *S. quelpaertensis* Nakai leaf extract and its fractions was assayed as described by Tottmar et al [15]. In brief, a sodium phosphate buffer (50mM), acetaldehyde (5mM), and NAD solution (0.5mM) were mixed together, and then 0.1mM ALDH solution (1 unit/mL) was added to the mixture. After adding the sample solution (200  $\mu$ L), the reactants were incubated for 10 minutes at 37°C prior to the measurement. Eventually, the absorbance at 370 nm was determined using a UV spectrophotometer. The control (100%) was prepared for comparison purposes to determine the value of the enhancement induced by the sample solutions on the ADH and ALDH activity assay.

### 3. Results

#### 3.1. Extraction yield

The extraction yields and actual dry weights after concentration are shown in Table 1. The aqueous fraction showed the highest extraction yield (33.23%), and the *n*-butanol fraction performed with the second strongest recovery rate (31.75%). Also, the *n*-hexane fraction exhibited a relatively high extraction yield (24.20%). However, compared to the fractions mentioned above, the chloroform fraction (4.71%) and the ethyl acetate fraction (6.11%) demonstrated relatively lower extraction yields. Among these fractions, the extraction yield of the chloroform fraction was the lowest, and the yield of the aqueous fraction was almost eight times that of the chloroform fraction.

#### 3.2. Total phenol content assay

The amounts of phenol content present in the *S. quelpaertensis* Nakai leaf extract and its fractions are shown in Table 2. The value of total phenol content varied, and ranged from 99.46 to 2197.09 mg GAE/g. Out of all of the other extracts and fractions, the ethyl acetate fraction had the highest rate ( $2197.09 \pm 93.75$  mg GAE/g). Following the ethyl acetate fraction, the *n*-hexane fraction ( $1588.08 \pm 28.79$  mg GAE/g), the *n*-butanol fraction ( $996.32 \pm 31.38$  mg GAE/g), 80% ethanol extract ( $784.05 \pm 4.99$  mg GAE/g), the aqueous fraction

( $304.37 \pm 2.62$  mg GAE/g), and the chloroform fraction ( $99.46 \pm 7.61$  mg GAE/g) exhibited the highest phenol contents. Furthermore, the solvents that demonstrated high polarity also had higher total phenol potential, indicating that the majority of phenolic compounds presented in *S. quelpaertensis* Nakai leaf extract and its fractions would be polar compounds and more attracted to high polarity solvents.

#### 3.3. DPPH radical scavenging activity assay

The strength of the sample solution that donated the hydrogen atom to the DPPH radical is shown in Table 2. As can be seen in the table, the DPPH radical scavenging activity varied from  $14.66 \pm 0.30$  to  $187.19 \pm 8.35$ . The highest radical scavenging activity was obtained with the ethyl acetate fraction ( $14.66 \pm 0.30$   $\mu$ g/mL), followed by the chloroform fraction ( $30.28 \pm 1.20$   $\mu$ g/mL), the *n*-butanol fraction ( $48.30 \pm 0.84$   $\mu$ g/mL), 80% ethanol extract ( $62.71 \pm 5.73$   $\mu$ g/mL), the *n*-hexane fraction ( $162.04 \pm 6.32$   $\mu$ g/mL), and the aqueous fraction ( $187.19 \pm 8.55$   $\mu$ g/mL). The chloroform and distilled water fractions had significantly lower activity than the ethyl acetate fraction. The ethyl acetate and *n*-hexane fractions showed much higher radical scavenging activity than BHT ( $42.02 \pm 0.97$   $\mu$ g/mL). However, none of the extracts and fractions exhibited stronger DPPH radical scavenging activity than  $\alpha$ -tocopherol ( $3.95 \pm 0.06$   $\mu$ g/mL).

#### 3.4. ABTS radical scavenging activity assay

As summarized in Table 2, the ABTS radical scavenging activity of *S. quelpaertensis* Nakai leaf extract and its fractions ranked in the same way as the values for DPPH radical scavenging activity, with the ethyl acetate fraction ( $29.55 \pm 0.92$   $\mu$ g/mL) as the lowest, then the *n*-hexane fraction ( $31.98 \pm 1.14$   $\mu$ g/mL), the *n*-butanol fraction ( $49.27 \pm 1.45$   $\mu$ g/mL), 80% ethanol extract ( $67.77 \pm 2.27$   $\mu$ g/mL), the chloroform fraction ( $187.48 \pm 6.69$   $\mu$ g/mL), and the distilled water fraction ( $196.06 \pm 4.67$   $\mu$ g/mL). Again,  $\alpha$ -tocopherol ( $4.99 \pm 0.42$   $\mu$ g/mL) showed a significantly higher value than any of the other extracts and fractions, and the ethyl acetate, *n*-hexane, and *n*-

**Table 1 – Extraction yields and obtained dry weights of *Sasa quelpaertensis* Nakai leaf extract and its partitioned fractions.**

Extract	Yield (%)	Weight (g)
80% Ethanol extract	9.26	331.73
<i>n</i> -Hexane fraction	24.20	80.29
Chloroform fraction	4.71	15.63
Ethyl acetate fraction	6.11	20.26
<i>n</i> -Butanol fraction	31.75	105.32
Aqueous fraction	33.23	110.23

**Table 2 – Assays of total phenol, DPPH, and ABTS radical scavenging activity for *Sasa quelpaertensis* Nakai leaf extract and its partitioned fractions.**

Extract	TPC (mg GAE/g) <sup>a</sup>	DPPH radical scavenging activity RC <sub>50</sub> ( $\mu$ g/mL) <sup>b</sup>	ABTS radical scavenging activity RC <sub>50</sub> ( $\mu$ g/mL) <sup>c</sup>
80% Ethanol extract	$784.05 \pm 4.99^d$	$62.71 \pm 5.73$	$67.77 \pm 2.27$
<i>n</i> -Hexane fraction	$1588.08 \pm 28.79$	$30.28 \pm 1.20$	$31.98 \pm 1.14$
Chloroform fraction	$99.46 \pm 7.61$	$162.04 \pm 6.32$	$187.48 \pm 6.69$
Ethyl acetate fraction	$2197.09 \pm 93.75$	$14.66 \pm 0.30$	$29.55 \pm 0.92$
<i>n</i> -Butanol fraction	$996.32 \pm 31.38$	$48.30 \pm 0.84$	$49.27 \pm 1.45$
Aqueous fraction	$304.37 \pm 2.62$	$187.19 \pm 8.55$	$196.06 \pm 4.67$
$\alpha$ -Tocopherol		$3.95 \pm 0.06$	$4.99 \pm 0.42$
Butylated hydroxytoluene		$40.02 \pm 0.97$	

ABTS = 2,2-azino-bis-(3-ethylbenzothiazothiazoline-6-sulfonic acid); DPPH = 1,1-diphenyl-1-picrylhydrazyl; GAE = gallic acid equivalent; TPC = total phenolic content.

<sup>a</sup> Total phenolic content analyzed in terms of the gallic acid equivalent (GAE) mg/g of extract; values are the average of the triplicates.

<sup>b</sup> RC<sub>50</sub>: Amount required for a 50% reduction of DPPH radicals after 30 minutes.

<sup>c</sup> RC<sub>50</sub>: Amount required for a 50% reduction of ABTS radicals after 20 minutes.

<sup>d</sup> Values are mean  $\pm$  standard deviation (SD) of duplicate determinations.



butanol fractions demonstrated relatively strong ABTS radical scavenging activity. However, the activities of the chloroform and distilled water fractions were five to nine times lower than those of the three fractions above.

### 3.5. Reducing power assay

The reducing capabilities of *S. quelpaertensis* Nakai leaves in 80% ethanol extract and each of the fractions were compared with the same concentration of  $\alpha$ -tocopherol as illustrated in Figure 1. The reducing capabilities of all extracts and fractions increased proportionally to the escalation of the concentrations. Ethyl acetate showed especially strong reducing capability in comparison to the other extracts and fractions for all concentrations. Although the ethyl acetate fraction had slightly lower capability than  $\alpha$ -tocopherol in 300  $\mu\text{g/mL}$ , its rates were much higher and stronger than  $\alpha$ -tocopherol in 100 and 200  $\mu\text{g/mL}$ . In the reducing power assay, in contrast to DPPH or ABTS radical scavenging activity, the *n*-hexane fraction exhibited the second lowest reducing power following the distilled water fraction. The chloroform fraction demonstrated the second highest power, followed by the *n*-butanol fraction and 80% ethanol extract.

### 3.6. Tyrosinase inhibitory activity assay

Figure 2 illustrates the percentage of inhibition of the two substrates, L-tyrosine and L-DOPA, to demonstrate the tyrosinase inhibitory activity ability. Among the extract and the five fractions, the highest tyrosinase inhibitory activity using L-tyrosine was obtained in the ethyl acetate fraction, whereas the second highest was in the chloroform fraction. Followed by the chloroform fraction, the order from highest to lowest was the *n*-butanol fraction, 80% ethanol extract, the *n*-hexane fraction, and the aqueous fraction. A dose-dependent manner was seen in all extracts and fractions. The *n*-hexane fraction demonstrated stronger inhibition against L-tyrosine than arbutin at low concentrations (63  $\mu\text{g/mL}$  and 125  $\mu\text{g/mL}$ ). Furthermore, the ethyl acetate, chloroform, and *n*-butanol fractions as well as 80% ethanol extract showed higher inhibitory activity than arbutin at all concentrations.

Similar to the case with L-tyrosine, significant tyrosinase inhibition was also observed with the use of L-DOPA as the substrate, as shown in Figure 2. As can be seen, the rates for all of the sample solutions show that tyrosinase inhibitory activity increased when more of the sample solution was added. Furthermore, its inhibitory activity was clearly enhanced as compared to the inhibition of L-tyrosine, meaning that the extract and the five fractions of *S. quelpaertensis* Nakai leaves inhibit more L-DOPA than L-tyrosine. As expected, the ethyl acetate fraction had higher rates than the extract and other fractions. The tyrosinase inhibitory activity was strongest to weakest in the following order: ethyl acetate > chloroform > *n*-butanol > *n*-hexane > aqueous > 80% ethanol extract. For L-DOPA, the arbutin rates were inferior to those for the extract and the fractions, and the aqueous fraction demonstrated relatively higher inhibitory activity. The results of these data may elucidate the high possibility of water-soluble active compounds to suppress L-DOPA in the aqueous fraction of *S. quelpaertensis* Nakai leaves.

### 3.7. ADH and ALDH inducing activity assay

How much the extract and each fraction induced the activity of ADH and ALDH against the control is displayed in Figure 3. In the ADH activity assay, the order from highest to lowest was as follows: the ethyl acetate fraction, the chloroform fraction, the *n*-butanol fraction, the *n*-hexane fraction, 80% ethanol extract, and the aqueous fraction. No increase was observed in 100  $\mu\text{g/mL}$  among all of the sample solutions. However, the ethyl acetate and chloroform fractions demonstrated approximately 40% and 20% enhancement, respectively, judging from the control in 200  $\mu\text{g/mL}$ . Moreover, in 300  $\mu\text{g/mL}$ , 80%, 70%, 40%, and 10% increases were seen in the ethyl acetate fraction, the chloroform fraction, the *n*-butanol fraction, the *n*-hexane fraction, and 80% ethanol extract, respectively. In contrast, the aqueous fraction exhibited significantly lower activity than the other fractions and the control, meaning that it did not exhibit any significant activity. The 80% ethanol extract and the *n*-hexane fraction had similar inducing abilities in 300  $\mu\text{g/mL}$  concentration.

The results of the ALDH activity assay (Figure 3) indicated that the rates for *S. quelpaertensis* Nakai leaf extract and its

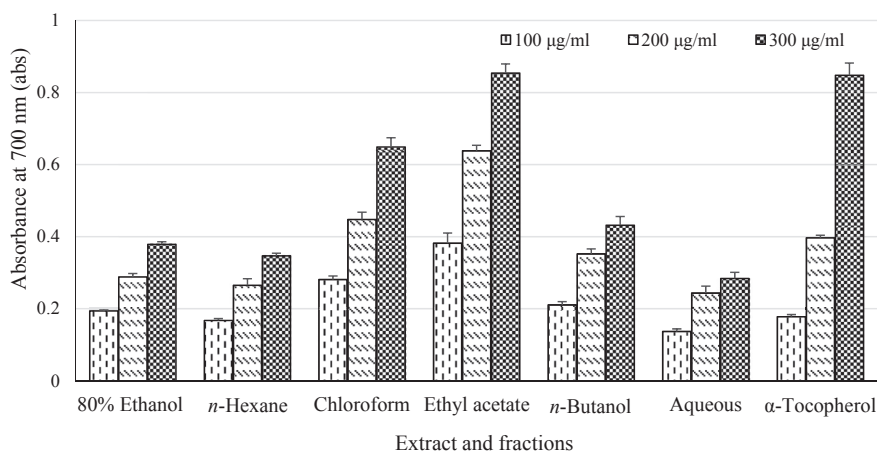
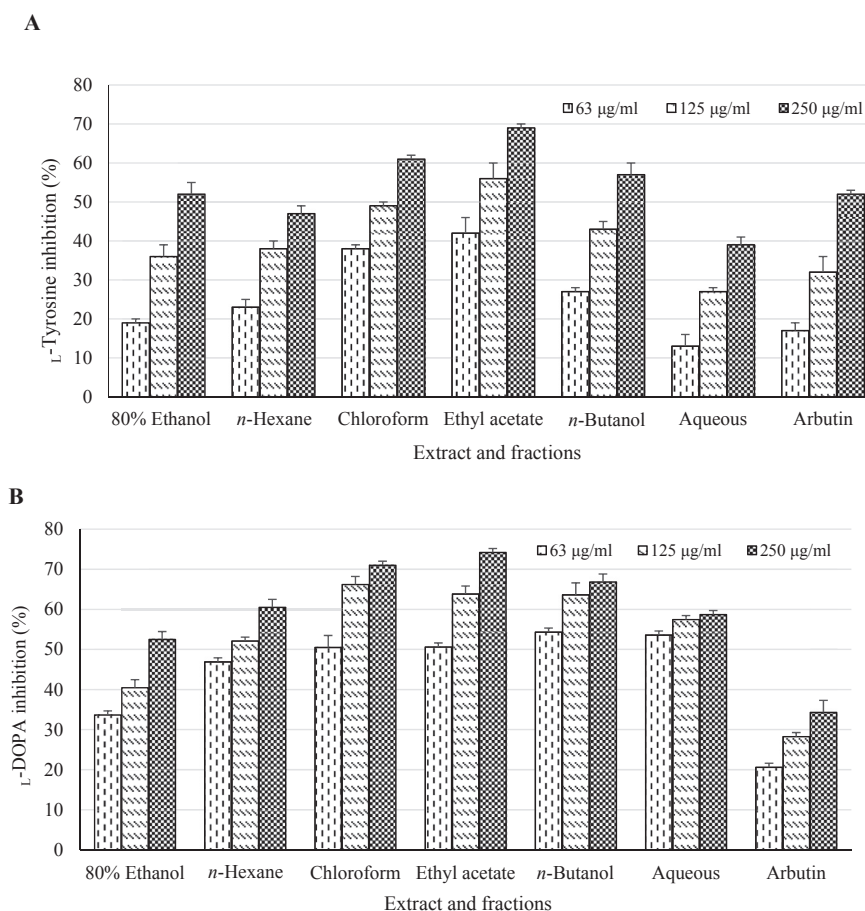


Figure 1 – The reducing power of *Sasa quelpaertensis* Nakai leaf extract and its partitioned fractions.



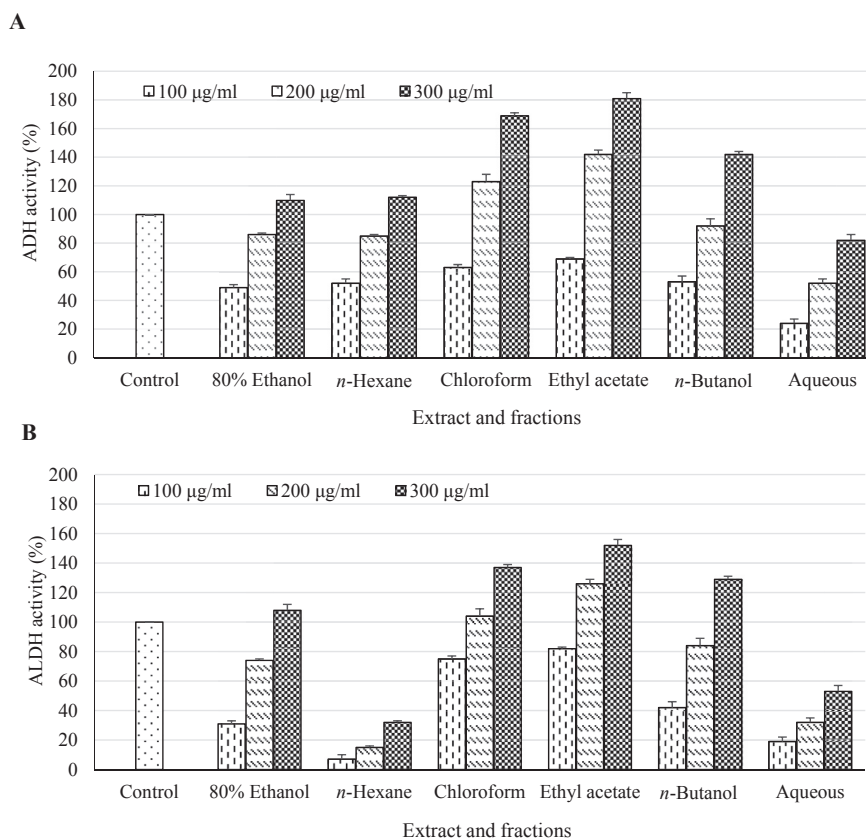
**Figure 2 – The tyrosinase inhibitory activity of *Sasa quelpaertensis* Nakai leaf extract and its partitioned fractions analyzed in terms of (A) L-tyrosine and (B) L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate.**

fractions were overall less effective than the rates for ADH activity. The decreasing order of efficiencies in the ALDH activity assay were almost similar to those for ADH activity, but the *n*-hexane fraction exhibited dramatically lower ability, and the aqueous fraction simultaneously showed much lower activity compared to that of ADH activity, indicating no inducement based on the control. However, the ethyl acetate fraction exhibited the highest rates among all extracts and fractions, followed by the chloroform fraction, the *n*-butanol fraction, and 80% ethanol extract, which also demonstrated higher enhancement. Our results showing that the *n*-hexane and aqueous fractions had lower potential could be explained by the presence of more nonsoluble active compounds.

#### 4. Discussion

Plants contain a variety of chemicals, including carbohydrates and phenolic acids. The former compound is what is called the “primary metabolite” or “central metabolite,” which mostly consists of hydrophilic substances and essential sources that plants need to maintain their vital bioprocesses such as growth or photosynthesis. In contrast, the latter compound is not directly involved in the above functions, but

is instead closely related to determining the plant's hue, defending it against any intruding enemy such as pests or pathogenic microorganisms, and working as a molecular response mechanism to conditions of extreme stress [16]. These compounds have been reported as possessing inner potential biological activity and extraction methods are widely used to retrieve them. Because each plant produces and accumulates a specific amount of active compounds in their tissues and attraction toward the solvents differs depending on its polarity, ideal extraction methods must be individually designed for optimization [17]. In this study, we partitioned five fractions (*n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous) from 80% ethanol extract to evaluate the extraction yield of *S. quelpaertensis* Nakai leaves. Table 1 shows the obtained dry weight and extraction yield of each extract and fraction, indicating that the aqueous, *n*-butanol, and *n*-hexane fractions demonstrated relatively high extraction yields. Conversely, the ethyl acetate and chloroform fractions exhibited lower extraction yields. Park and Lim [18] stated in their study on *S. borealis*, which is a part of the same family and closely related to *S. quelpaertensis* Nakai, that a high polarity solvent such as the aqueous ( $5.1 \pm 0.5\%$ ), and *n*-butanol fractions ( $1.3 \pm 0.2\%$ ) actually produced higher yields than the chloroform ( $1.1 \pm 0.2\%$ ) and ethyl acetate fractions



**Figure 3 – (A) ADH and (B) ALDH activity of *Sasa quepaertensis* Nakai leaf extract and its partitioned fractions. ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase.**

( $0.6 \pm 0.1\%$ ). These findings correspond with those of our study. However, in another study, an orange peel directly extracted with methanol, ethanol, dichloromethane, acetone, *n*-hexane, and ethyl acetate, the *n*-hexane extract (8.27%) demonstrated the lowest extraction yield, and the ethyl acetate extract (24.92%) exhibited the highest yield [19]. Based on this comparison, the extraction process could be one factor contributing to the low extraction yield for the ethyl acetate and chloroform fractions. Furthermore, when it comes to the extract (80% ethanol), our rates were much lower than those in the investigation of Yang et al [20], whose 80% ethanol extract of an entire Cortex Moutan plant yielded  $24.73 \pm 0.39\%$ , demonstrating a higher yield than the results reported in some of the other published literature [21]. As Stalikas [22] indicated, the extraction yield is significantly affected not only by the solvent's polarity but also by other factors including the types of plant parts, storage times, and temperature. Although there might be many factors that need to be considered, the prominent difference from other literature could likely be explained by the different extraction method (directly extracted or partitioned), the kind of plant, or the intrinsic substances that were accumulated in the plant tissues.

Phenolic compounds can be found ubiquitously in living organisms, and plants possess suitable amounts of them

inside their elongated bodies. Phenolic compounds usually exist in diverse forms, such as hydroxycinnamic acids (phenolic acids), hydrolyzable and condensed tannins (polyphenol), and flavonoids [23]. Through the extensive research on phenolic compounds over the years, scientists have found remarkable biological properties in terms of antioxidant activity, anti-inflammatory activity, anticancer activity, and antimicrobial activity, among others [24]. Because of the noteworthy effects on human health, ways in which these compounds can be used as a part of our daily diet have been receiving more interest in recent years [25]. As the results provided in Table 2 reveal, significantly higher amounts of phenolics were found in the ethyl acetate and *n*-hexane fractions. The *n*-butanol fraction also showed a slightly higher total phenol content, followed by the 80% ethanol extract, the aqueous fraction, and the chloroform fraction. In general, the crude extract, the ethyl acetate fraction, and the *n*-butanol fraction were more effective for retrieving phenol compounds based on previous research [10], and our study was in agreement with the results in this literature. Considering the low extraction yield and high total phenol content of the ethyl acetate fraction, it could be predicted that phenol compounds mostly occupy the ethyl acetate fraction as compared to the other fractions, or some phenolic entity reacts strongly with the Folin–Ciocalteu reagent, resulting in a solution more

deeply discolored than other phenolic molecules [26]. On the contrary, the *n*-hexane fraction demonstrated extremely high total phenol content in this study, which differentiated our investigation from other studies [27]. Generally, molecules with lower polarity have a propensity to be dissolved more effectively with lower polarity solvents. In *S. quelpaertensis* Nakai leaves, lower polarity molecules may exist in high concentrations and therefore contribute to greater biological activity.

The DPPH radical scavenging activity assay is probably the most commonly used method among other antioxidant evaluating assays for natural compounds. The DPPH radical is a free radical that has nitrogen in the center of its structure. The basic principle involves the donation of the hydrogen atom to the unpaired nitrogen molecule in the DPPH radical, creating corresponding hydrazine [28]. The deep purple chromogen radical is reduced by the antioxidant in the sample solution and turned into a light yellow color (1,1-diphenyl-2-picrylhydrazine). The reduced absorbance is usually measured between the 515–520 nm range [29]. In this assay, we used a 96-well microplate with methanolic DPPH solution. The radical scavenging activity varied significantly depending on the solvent used in this assay. The  $RC_{50}$  value for the ethyl acetate fraction was  $14.66 \pm 0.30$   $\mu$ g/mL, and the values for the *n*-hexane fraction, the *n*-butanol fraction, and 80% ethanol extract were  $30.28 \pm 1.20$ ,  $48.30 \pm 0.84$ , and  $62.71 \pm 5.73$   $\mu$ g/mL, respectively (Table 2). These four fractions demonstrated extremely high to mild DPPH radical scavenging activity, but the chloroform and aqueous fractions exhibited relatively lower activity than the fractions above. Based on Table 2, the extract and fractions that possess the highest total phenol content tend to show stronger DPPH radical scavenging activity. Kim and Lim [30] suggested that the overall antioxidant ability of sample solutions mainly depends on the amount of total phenol content in each extract and fraction. In our study, it is clear that the total phenol content is highly correlated with DPPH radical scavenging activity. In addition, Yokozawa et al [31] reported the scavenging ability of phenolic compounds against the DPPH radical to be closely associated with their chemical structure. The existence of the hydroxyl group in the C-3 position greatly affected the radical scavenging activity in contrast to the C-5 position. The fractions that showed stronger DPPH radical scavenging activity might therefore contain a lot of phenolic compounds that are structurally effective for scavenging the DPPH radical. Moreover, it is interesting to note that the *n*-hexane fraction exhibited the second highest radical scavenging activity. This could indicate that the *n*-hexane fraction of *S. quelpaertensis* Nakai leaves holds not only abandoned low-polarity molecules, but that these molecules function as an active agent in scavenging unpaired DPPH radicals.

The ABTS radical scavenging activity is another common method in addition to DPPH radical scavenging activity that can be used to measure the antioxidant ability of sample solutions. Both DPPH and ABTS radicals are stable and show strong absorption around 510 and 730 nm, respectively. The ABTS radical is generated from ABTS oxidation with potassium persulfate, and when the ABTS radical is scavenged by antioxidants, its color is lightened from a deep green color [32]. As presented in Table 2, the order of ABTS radical

scavenging activity was exactly the same as the order of DPPH radical scavenging activity, which was the ethyl acetate fraction first, followed by the *n*-hexane fraction, the *n*-butanol fraction, 80% ethanol extract, the chloroform fraction, and the aqueous fraction. Consequently, the results of our study revealed that the phenolic compounds are somehow associated with the ability of ABTS radical scavenging, which was also described in the study by Yang et al [32]. Furthermore, although both assays use similar mechanisms for mediating the radical, the DPPH radical scavenging activity assay is more selective, meaning that it tends to react more with the phenolic compounds that belong to the high-reacting hydroxyl group. The ABTS radical scavenging activity assay, by contrast, also reacts with the phenolic compounds that the DPPH radical scavenging activity assay cannot detect [33]. Yu et al [26] discussed the different order of DPPH and ABTS radical scavenging activities for four readily available cereal products, and suggested that the slightly different chemical mechanisms of the two assays and the different chemical properties of the two radicals caused this visible difference. Even if there is no significant difference recognized between the two assays in our study, the ABTS radical scavenging activity assay still demonstrated slightly lower values than the DPPH radical activity assay. It could therefore be suggested that nonactive phenolic compounds, which were not detected by the DPPH radical scavenging assay, reacted in the ABTS radical assay and therefore lowered its values.

The reducing ability of the sample in itself is not directly associated with its antioxidant ability, but the reducing power assay is frequently used as a parameter to determine the electron transferring capability, which is considered to be an important antioxidant mechanism of antioxidants, including phenolic compounds [34]. When using the reducing power assay, the  $Fe^{3+}$  (potassium ferricyanide) and ferric chloride are transformed into  $Fe^{2+}$  (potassium ferrocyanide) and ferrous chloride by the antioxidants present in the extract and the fractions, which have a maximum absorption at 700 nm [35]. As shown in Figure 1, and as expected, the ethyl acetate fraction was able to reduce  $Fe^{3+}$  into  $Fe^{2+}$  in high amounts, whereas the reducing power of the aqueous fraction was lower than that of the extract and other fractions. Regardless of the generally low activity in the radical scavenging activity assays, the chloroform fraction demonstrated the second highest reducing ability. In this regard, the chloroform fraction possessed a small amount of low-active hydrogen and electron donor, but had relatively high amounts of reducing agents. This result is in accordance with a report indicating that chloroform fraction had the highest effect on superoxide dismutase (SOD)-like and reducing power activity, even though low activity was found in the DPPH radical scavenging activity [36]. Conversely, the *n*-hexane fraction showed relatively lower reducing ability considering the higher DPPH and ABTS radical scavenging activity in Table 1. This likely indicates that the substances that excel in reducing the free radical would be mostly nonwater-soluble, and the high amount of total phenol content in the *n*-hexane fraction detected in this study would be a less-active reducing agent. Furthermore, Hyun et al [37] found that the ethyl acetate fraction worked effectively as a stronger reductant than an extract and other fractions (methanol extract with *n*-hexane,



*n*-butanol, and aqueous fractions) on Korean crow berry (*Empetrum nigrum* var. *japonicum*). A similar result was also observed in a study by Hyun et al [10], whose observations revealed that the ethyl acetate fraction of *Thymus quinquecostatus* Celak exhibited the highest reducing ability, therefore leading to the conclusion that phenolic compounds play major roles as antioxidants. In our study, the ethyl acetate fraction showed the highest total phenol content as well as the highest value for the reducing power assay; it can therefore be inferred that phenol compounds are somehow involved in the overall reducing ability.

Melanin can be found in many organisms, including humans, plants, and microorganisms, and it is deeply involved in determining the color of the human skin, hair, and the iris in the eyes. Melanin is catalyzed from tyrosine, which is then changed by tyrosinase into L-DOPA. L-DOPA is further oxidized and eventually forms melanin after undergoing several complex biological steps. Even though melanin is an essential biological pigment for most living organisms, its surplus can lead to various pathologies, including seborrheic dermatitis, keratosis, melasma, and malignant melanomas. As a remedy for a surplus, because tyrosinase plays a key role in synthesizing melanin, tyrosinase inhibitors such as kojic acid and hydroquinone have been used in the cosmetic and medical fields in the past decade. Simultaneously, the development of safe and effective natural tyrosinase inhibitors has become an important topic of investigation in recent years because of the benefits that plants originally have as intrinsic substances [38]. As shown in Figure 2, the ethyl acetate fraction was found to have the highest tyrosinase inhibitory activity with both L-tyrosine and L-DOPA used as the substrate. In the case of the chloroform and *n*-butanol fractions, the tyrosinase inhibitory activity was found to be slightly higher for the chloroform fraction than the *n*-butanol fraction. In a study conducted by Choi et al [39], the strong correlation between total phenol content and tyrosinase inhibitory activity was highlighted. Kaempferol, ellagic acid, and quercetin—which are all phenolic compounds isolated from rose oil distillation waste water—exhibited stronger tyrosinase inhibitory potential than kojic acid, which was used as reference [40]. These reports partly support our study, because the ethyl acetate and *n*-butanol fractions demonstrated higher tyrosinase inhibitory activity as well as higher total phenol content. Moreover, L-tyrosine was a little less inhibited in comparison to L-DOPA. This could mean that the active compounds inhibiting L-DOPA would be contained in relatively high amounts in *S. quelpaertensis* Nakai leaves. However, the chloroform fraction exhibited the second highest tyrosinase inhibitory activity in spite of its lowest total phenol content. Similar to the case with the reducing power assay, this clearly demonstrates that phenolic compounds are not the only active compounds present in *S. quelpaertensis* Nakai leaves.

Alcohol has been consumed since 7 B.C., with its use first recognized in China for many reasons including relieving thirst and religious ceremonies. The main component of alcohol is ethanol, and when ingested, it is processed mostly in the liver by two unique enzymes called ADH and ALDH. ADH oxidizes ethanol into acetaldehyde, a highly volatile substance that is more toxic than ethanol, and then

acetaldehyde is immediately catalyzed by ALDH into water and acetic acid. Eventually, acetic acid is further detoxified into carbon dioxide. This hepatic oxidation, however, would not work properly in cases of chronic overdrinking of alcohol, which would increase the risk of inducing free radicals, fatigue, vomiting, diarrhea, and nausea [41]. In this study, we investigated how effective the extract and fractions of *S. quelpaertensis* Nakai leaves are for inducing ADH and ALDH activity compared with a control of 100% (without taking a sample solution). Compared with the control, the ethyl acetate fraction showed the highest activity with approximately 80% and 50% inducing activity for ADH and ALDH, respectively (Figure 3). The chloroform and *n*-butanol fractions also exhibited higher inducing activity, whereas the *n*-hexane and aqueous fractions did not demonstrate any enhanced activity toward ADH and ALDH. Nonplant-derived material (rock fish, *Sebastes schlegeli*) extracted with distilled water also showed lower inducing activity for both ADH and ALDH in another study [14], which is similar to the results of our study. However, according to study by Kang et al [42], an aqueous fraction partitioned from methanol extract enhanced ADH activity more than acidic, base, neutral, and phenolic fractions of three vegetables (*Glycine max*, *Oenathe javanica*, *Raphanus sativus* var. *hortensis* for. *acanthiformis*), and the suggested apoenzyme, which is both essential for ADH activity and nonwater-soluble, did not work properly because of the strong acidic and base conditions. In our study, apoenzyme might bind with ADH in organic solvents, showing higher induction without being affected by high and low pH levels. In another study, amino acids such as arginine, aspartic acid, glutamic acid, alanine, and methionine played more significant roles than the phenolic compounds in enhancing ADH and ALDH activity [43]. Although the fractions possessing higher phenol content exhibited higher ADH and ALDH activity (except for the *n*-hexane and chloroform fractions), phenolic compounds might not be the best substances to induce ADH and ALDH activity. To identify the active compounds for inducing ADH and ALDH, more study and investigation would be necessary.

## 5. Conclusions

The extract (80% ethanol) and its five partitioned fractions (*n*-hexane, chloroform, ethyl acetate, *n*-butanol, aqueous) of *S. quelpaertensis* Nakai leaves exhibited various levels of biological activity in antioxidant, tyrosinase inhibitory, and ADH and ALDH activity assays. The outcome revealed that the ethyl acetate fraction demonstrated the highest activity, and the *n*-butanol fraction also showed high activity in all of the assays. The *n*-hexane fraction had the second highest values for the DPPH and ABTS radical scavenging assays as well as for total phenol content, but it exhibited relatively lower activity in the other assays. This is likely indicative of the *n*-hexane fraction containing active compounds that more effectively scavenge the free radicals. The chloroform fraction, by contrast, had the opposite values of the *n*-hexane fraction, suggesting that it contained higher reducing, tyrosinase inhibitory, and ADH and ALDH inducing agents. Our findings strongly support that *S. quelpaertensis* Nakai leaves can be a

good candidate for use in dietary foods, drugs, and cosmetics. We have provided basic information relating to several *in vitro* assays; however, identifying the active compounds and performing more biological and chemical assays would be necessary for a better understanding of its application in industrial purposes.

## Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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