

Chemical Characterization of Neutral Extracts Prepared by Treating *Pinus radiata* Bark with Sodium Bicarbonate¹

Ji Sun MUN² · Hwan Chul KIM² · Sung Phil MUN^{3,†}

ABSTRACT

A neutral extract (NE), that is soluble in cold water and has excellent antioxidant activity, from *Pinus radiata* pine bark was prepared by sodium bicarbonate treatment, and its chemical characteristics were investigated. NE was prepared by treating *P. radiata* bark with 0.8% NaHCO₃ aqueous solution with a 5 : 1 liquor-to-bark ratio at boiling temperature for 1 h, resulting in 44% yield and final pH of 6.66. The yield of NE was 11% higher than that of the hot water extract (HWE) due to the increase in the solubility of polyphenols, the main component in the bark, by NaHCO₃ treatment. NE was characterized through FT-IR, NMR, and MALDI TOF MS analyses. The results indicated that NE is mostly composed of proanthocyanidins (PAs) consisting of procyanidin (PC) units. The acetylated neutral extract (Ac-NE) had weight average molecular weight (\overline{M}_w) of 5,300 Da. The Ac-NE had wide molecular weight distribution and its polydispersity ($\overline{M}_w/\overline{M}_n$) was 6 times higher than that of pure PA. The antioxidant activity of NE was determined by 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and showed that NE had comparable antioxidant activity with pure PA.

Keywords: *Pinus radiata* bark, neutral extract (NE), sodium bicarbonate, proanthocyanidins (PAs), antioxidant activity

1. INTRODUCTION

In 2019, over 85% of wood was imported to Korea, of which timber imports accounted for around 10%. About 75% of the imported timber is *P. radiata* which came from New Zealand with an import volume of approximately 2 million m³ (KFS, 2020). *P. radiata* wood is currently used in various fields such as construction, packaging, furniture and joinery, as well as in the production of plywood and medium-density fiberboard (Bayne, 2015). *P. radiata* bark accounts for

10 – 12% of total timber (Murphy and Cown, 2015). Taking this into consideration, about 200,000 m³ of bark per year is discharged from wood and its related industries in Korea. The bark is currently used as fuel, compost, and landscaping products (Murphy and Cown, 2015).

The bark of *P. radiata* is a rich source of proanthocyanidins (PAs, Fig. 1) (Ku *et al.*, 2011), a potent antioxidant (Prior and Gu, 2005; Ku and Mun, 2008; Ku *et al.*, 2011; Mun, 2014). Studies of pine bark extracts have been conducted and the extracts have shown anti-

¹ Date Received October 7, 2020, Date Accepted October 29, 2020

² Department of Carbon Materials and Fiber Engineering, Jeonbuk National University, Jeonju 54896, Republic of Korea

³ Department of Wood Science and Technology, Jeonbuk National University, Jeonju 54896, Republic of Korea

† Corresponding author: Sung Phil MUN (e-mail: msp@jbnu.ac.kr, ORCID: 0000-0002-3475-4965)

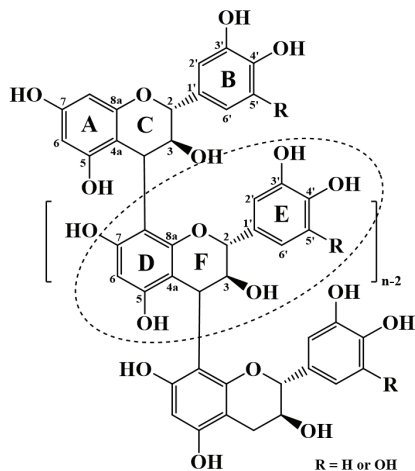


Fig. 1. Chemical structure of proanthocyanidin (PA) isolated from *P. radiata* bark.

hepatotoxic, anticarcinogenic, antioxidant, and anti-inflammatory effects (Rohdewald, 2002; Kim and Park, 2004; Ku and Mun, 2008). For that reason, pine bark extracts are sold worldwide as health supplements and cosmetic additives under the name of Pycnogenol[®], Enzogenol[®], and Pinoradiol[®] (Packer *et al.*, 1999; Ghosh and Mukherjee, 2009; Choi *et al.*, 2018). PA-rich extracts from *P. radiata* bark have also shown the potential as a termite deterrent (Mun and Nicholas, 2017) and as an adhesive for particle board production (Pizzi, 1982).

Bark extraction can be conducted in alkaline, cold water, hot water, organic solvents, and neutral conditions. The bark of *P. radiata* is easily extracted in highly alkaline conditions, but severe chemical modifications of the polyphenols occur and the solubility of the alkaline extracts are greatly decreased due to the formation of phlobaphenes by the rearrangement of bark polyphenols such as PA (Sealy-Fisher and Pizzi, 1992; Jeong and Mun, 2018). Hot water extraction is commonly used because PAs, which are a major component in most pine barks, have

higher solubility in hot water than cold. Furthermore, hot water extraction is environmentally safer than using organic solvent extraction. However, when hot water extracts (HWE) are cooled down, precipitation occurs. In addition, after drying, when powdered extracts are dissolved in water again, many insoluble portions generated by the association between PA molecules make the use of these products very difficult (Mun and Lim, 2008).

On the other hand, Mun and Lim reported that when *P. radiata* bark is extracted using a weak alkali, such as Na₂CO₃ and NaHCO₃, and the final extraction pH becomes neutral, extract yields are higher than with hot water. The extracts do not precipitate even after cooling and also dissolves well after drying. They also reported that the extracts retain strong antioxidant properties similar to pure PA (Mun and Lim, 2008). Since the weak alkaline extraction of *P. radiata* bark gave higher extract yields than HWE, this extraction would be economical. In addition, the extracts had high antioxidant activity which would be beneficial to health supplement, functional cosmetic, and natural dyestuff industries. However, in the previous study, the chemical characterization of NE was performed partially, thus, a more detailed analysis and information on NE should be needed for further utilization of *P. radiata* bark. Therefore, in this study, *P. radiata* bark was extracted with NaHCO₃ aqueous solution to prepare NE and the chemical characteristics of NE were investigated in comparison with pure PA prepared from the same pine bark.

2. MATERIALS and METHODS

2.1. Materials

The bark used in the experiment was kindly provided by Baeksan Wood Co., Ltd. (Gunsan-si, South Korea) and the age of the *P. radiata* wood was 70

- 80 years old. After removing the scales and inner bark, the remainder was crushed with a hammer and then ground with a blender (Primaire, Hanil, South Korea). The ground bark was sieved through 40 mesh. The powder that passed through the mesh was stored in a zipper bag at room temperature and was used as the material for this study.

Sodium bicarbonate (NaHCO₃, 99.5%), acetic anhydride (93.0%), tetrahydrofuran (THF, 99.9%), and methanol (MeOH, 99.9%) were purchased from Duksan Pure Chemical Co., Ltd. (South Korea), pyridine (99.5%) from Kanto Chemical (Japan), 2-diphenyl-1-picrylhydrazyl (DPPH) radical from Sigma- Aldrich (Germany), deuterated acetone (acetone-*d*₆) and deuterium oxide (D₂O) from Merck KGaA (Germany).

2.2. Chemical composition of *P. radiata* bark

The ash, cold water, hot water, 1% NaOH, alcohol-benzene extract, and total lignin content of the *P. radiata* bark were measured in accordance with TAPPI test methods (T 204, 2007; T 207, 2008; T 211, 2002; T 212, 2002; T 222, 2006). However, the lignin content of the pine bark obtained *via* Klason method was higher than the accepted values because of the presence of polyphenols. Thus, in this study, the bark polyphenols were extracted with 1% NaOH, and thereafter the polyphenols-corrected lignin value was determined using the residues following 1% NaOH extraction.

2.3. Preparation of NE

A 100 g (o.d.) of the bark powder (40 mesh passed) and 500 mL of 0.8% NaHCO₃ aqueous solution were added to a 1 L round bottom flask. The flask was fitted to a condenser and then placed in a PEG #400 bath preset at 110°C. The extraction was carried out for 1 h from the start of boiling with occasional shaking. After the extraction, the bark slurry was fil-

tered through a 25G3 glass filter (Pyrex, UK), and the residue was washed with 500 mL of hot distilled-deionized water (DI-water). After washing, the glass filter was dried in a 105°C convection oven (FO-600M, Jeio Tech, South Korea) for 48 h. The yield of NE was then calculated as follows (1):

$$\begin{aligned} \text{Yield of NE (\%)} \\ = [\text{BP (o.d.)} - \text{RE (o.d.)}]/\text{BP (o.d.)}] \times 100 \quad (1) \end{aligned}$$

where, BP is bark powder (g), o.d. is oven dried weight, and RE is residue after extraction.

After cooling NE, an aliquot was taken into a 30 mL conical beaker and the pH was measured by pH meter (CyberScan pH 1500, Thermo, USA). The NE was equally transferred into four 1 L eggplant flasks. The extracts in the flasks were frozen by carefully rotating the flasks at -35°C in a low temperature circulation bath (RBC-30, Jeio Tech, South Korea). The flasks were lyophilized for 24 h in a freeze dryer (FDU-540, Eyela, Japan). The dried extracts in the flasks were transferred into a 950 mL plastic bottle and then vacuum dried for 80 h at room temperature in the presence of P₂O₅.

2.4. Acetylation of NE

A 500 mg of the thoroughly dried NE and 10 mL of anhydrous pyridine were added into a 50 mL Erlenmeyer flask. The flask was sonicated for 90 s to disperse the mixture. Afterwards, 10 mL of acetic anhydride was added and then stirred with a magnetic stirrer (SR-306, Advantec Toyokaisha, Japan) for 48 h at room temperature. After 48 h, 175 g of crushed ice and 200 g of DI-water were added into a 500 mL beaker and was stirred vigorously with a magnetic stirrer (RCN-7, Eyela, Japan). The acetylated sample was sprayed into the beaker by using a tapered pipette and allowed to stir for 1 h at room temperature. The acetylated precipitates were collected by filtration using a

nylon 66 membrane filter (47 mm diameter, 0.45 μm , Alltech, USA). The precipitates on the filter were washed with 300 mL of DI-water. The filter was placed in an aluminum dish and then vacuum dried for 24 h in the presence of P_2O_5 .

2.5. Instrumental analysis of NE

2.5.1. Gel Permeation Chromatography (GPC)

A 2 mg of the acetylated NE (Ac-NE) and 1 mL of THF were added into a 10 mL conical beaker. The beaker was sonicated for 10 s for complete dissolution and then filtered through a 0.45 μm nylon syringe filter (Phenomenex, Torrance, CA, USA). The filtrate was transferred into a 2 mL vial and subjected to GPC. The acetylated pure PA (Ac-pure PA) prepared from *P. radiata* bark was used in this experiment as a sample for comparison. The GPC was conducted under the conditions shown in Table 1. The molecular weight (MW) of the samples was calculated from the standard curves prepared with phenol and polystyrene standards.

2.5.2. Fourier Transform–Infrared Spectroscopy (FT–IR)

The analysis was performed using the ATR method at 400 – 4000 cm^{-1} with an FT-IR spectrophotometer (Frontier, Perkin Elmer, USA) in the Center for University-Wide Research Facilities (CURF) at Jeonbuk National University. The measurement was conducted under

conditions of 20°C with 44% relative humidity.

2.5.3. ^{13}C Nuclear Magnetic Resonance (^{13}C NMR)

A 100 mg of the Ac-NE, 0.4 mL of D_2O , and 0.2 mL of acetone- d_6 were added into a 10 mL conical beaker. The beaker was sonicated for 1 min to completely dissolve the sample. The solution was passed through a filter formed by placing a fine glass wool for GC inside a Pasteur pipette and the filtrate was immediately transferred into an NMR tube. The conical beaker was washed with additional D_2O and acetone- d_6 in a 2 : 1 (v/v) ratio. The solution was transferred onto the filter to dissolve the remaining part in the filter and was received into the NMR tube again. The measurement was conducted with a 600 MHz FT-NMR spectrometer (JNM-ECA600, JEOL Ltd., Japan) in the CURF at Jeonbuk National University.

2.5.4. Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI TOF MS)

Both Ac-pure PA and NE samples after GPC analysis were also used for MALDI TOF MS analysis. A 10 mg of NaCl and 10 mg of 2,5-dihydroxybenzoic acid (DHB) matrix were dissolved in 1 mL of 50% acrylonitrile containing 0.1% trifluoroacetic acid. The sample solution, NaCl solution, and DHB were mixed

Table 1. Analysis conditions for GPC

Analyzer	GPC configuration: LC-20AD (pump) + SPD-M20A (detector) + CTO-20A (column oven), Shimadzu, Japan
Column	PLgel 10 μm MIXED-B (300 \times 7.5 mm, AGILENT, USA)
Flow rate	0.5 mL/min
Sample injection volume	10 μL
Eluent	THF
Column oven temperature	30°C
Detector	UV (254 nm: polystyrene standards, 280 nm: samples and phenol)
Analysis time	30 min

in the ratio of 1 : 1 : 1, and a 1 μ L aliquot sample was used for the analysis. The mixture was placed on a MALDI target, and the solvent was air dried. The measurement was conducted with MALDI TOF MS (Applied Biosystems VOYAGER-STR MALDI TOF MS, USA) at Korea Basic Science Institute (Jeonju).

2.6. Antioxidant activity

The antioxidant activity of the NE was determined by DPPH free radical scavenging assay. The DPPH solution was prepared by dissolving 1.78 mg of DPPH in 30 mL MeOH in a 50 mL Erlenmeyer flask, and the flask was covered with aluminum foil. A 5 mg of NE and 25 mL MeOH were added to another 50 mL Erlenmeyer flask. The flask was sonicated for 1 min and then allowed to stand for 30 min at room temperature. This solution was considered the stock solution. Various concentrations of solutions were made by diluting the stock solution with MeOH. The pure PA sample was prepared with the same method for comparison. Triplicate sets of 150 μ L diluted solutions were placed in a 96-well plate (Corning Incorporated, USA) using a multi- pipette (Eppendorf, Germany) and 150 μ L of DPPH solutions were added. The blank consisted of 300 μ L of MeOH, and the control was 150 μ L of DPPH and 150 μ L of MeOH. The plate was left in a microplate reader (ELx808, Bio-Tek, USA) set at 28 $^{\circ}$ C for 30 min. The measurement was conducted at an absorbance of 515 nm. DPPH free radical scavenging activity was calculated as follows (2):

$$\begin{aligned} \text{DPPH free radical scavenging (\%)} \\ = [(A_0 - A_1)/A_0] \times 100 \end{aligned} \quad (2)$$

where, A_0 is absorbance of the control and A_1 is absorbance of the sample.

3. RESULTS and DISCUSSION

3.1. Chemical composition of *P. radiata* bark

Analysis of ash, extracts, lignin, and polyphenol content was performed to determine the chemical composition of *P. radiata* bark and the results are shown in Table 2. The content of ash, cold-water, hot-water, and alcohol-benzene extracts was 0.42%, 20.45%, 32.81%, and 12.69%, respectively. The yield of the alkaline extract was more than 70%, significantly higher than that of cold-water, hot-water, and organic solvent extracts as reported by Mun and Lim (2008). In contrast, the majority of the polyphenols in *P. radiata* were extracted by 1% NaOH (Jeong and Mun, 2018). Hence, the 1% NaOH extracts can be used as an estimate of the total polyphenolic content. From the calculation, the total polyphenol content was 53.94% corresponding to around 74% of the 1% NaOH extract.

3.2. Characterization of NE from *P. radiata* bark

When *P. radiata* bark was extracted with 0.8% NaHCO₃

Table 2. Chemical composition of *P. radiata* bark

Ash (%)	0.42 \pm 0.00
Extracts (%)	
Cold-water	20.45 \pm 0.38
Hot-water	32.81 \pm 0.05
1% NaOH	72.85 \pm 0.39
Alcohol-benzene	12.69 \pm 0.40
Lignin (% on bark) before 1% NaOH extraction	
Klason	66.32 \pm 0.21
Acid-soluble	14.30 \pm 0.20
Total	80.62
Lignin (% on bark) after 1% NaOH extraction	
Klason ²	12.38 \pm 0.07
Acid-soluble	0.11 \pm 0.00
Total	12.49
Total polyphenol (%)	53.94

Total polyphenol (%) was calculated by subtracting the Klason lignin after 1% NaOH extraction from the Klason lignin before 1% NaOH extraction.

aqueous solution, the yield of the bark extracts and their pH were 44% and 6.66, respectively. As shown in Table 2, the yield of HWE from *P. radiata* bark was around 33%. However, when this bark was extracted with weak alkali to attain neutral pH, the yield increased by more than 10%. This was thought to be due to the increased solubility of bark polyphenols such as PAs in weakly alkaline conditions. The chemical structures and composition of NE were examined through GPC, FT-IR, ^{13}C NMR, and MALDI TOF MS analyses.

3.2.1. GPC

GPC of Ac-NE and Ac-pure PA was performed to determine the number average molecular weight (\overline{M}_n), weight average molecular weight (\overline{M}_w), and polydispersity ($\overline{M}_w/\overline{M}_n$). Fig. 2 shows the MW distribution of Ac-pure PA and Ac-NE. The high and low MW fractions of NE were more widely distributed than those of pure PA. In addition, the peak MW of NE was higher than pure PA.

Table 3 shows the values for \overline{M}_n , \overline{M}_w , and polydispersity of Ac-pure PA and Ac-NE. For Ac-NE, the

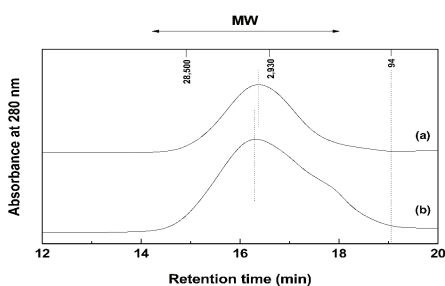


Fig. 2. MW distribution of Ac-pure PA (a) and Ac-NE (b); MW standards, phenol (94), and polystyrenes (2930, 28500, 148000, 841700, 7500000).

Table 3. Average MW and polydispersity of Ac-pure PA and Ac-NE

Sample	\overline{M}_w	\overline{M}_n	$\overline{M}_w/\overline{M}_n$
Ac-pure PA	5897	1854	3.2
Ac-NE	5289	286	18.5

\overline{M}_w was about 600 Da lower and the n was 1/6 that of Ac-pure PA because the MW of Ac-NE was widely distributed from high to low MW fractions as shown in Fig. 2. Therefore, it was clear that a broad range of UV-absorbing materials of different molecular sizes were extracted from the bark since the polydispersity of NE was 6 times higher than that of pure PA.

3.2.2. FT-IR

The FT-IR analysis was performed to determine the functional groups in NE. Fig. 3 shows the FT-IR spectra for pure PA and NE. The overall FT-IR spectrum pattern of NE was similar to that of the pure PA prepared from the same pine bark. The characteristic bands of the aromatic rings appeared at 1605, 1520, and 1450 cm^{-1} with the strong and broad stretching bands of the -OH group of polymeric phenol at 3300 cm^{-1} in both spectra. Also, characteristic bands of typical polyflavonoids were present including symmetric stretching bands of ether groups (=C-O-C bonds) as shown at 1067 cm^{-1} (Yazaki and Hillis, 1977). This confirmed the presence of PAs in NE. In addition, NE showed a single band at 1520 cm^{-1} and 779 cm^{-1} like pure PA. This suggested that NE was mainly composed of procyanidin (PC) structure of PAs.

The strength of the 1067 cm^{-1} absorption band in NE was higher than that of the pure PA. The strong C-O single-bond stretching vibrations were observed in the range from 1260 to 1000 cm^{-1} . The spectrum

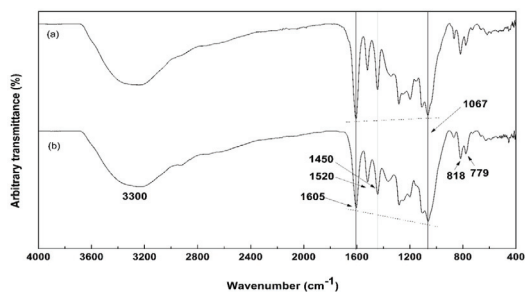


Fig. 3. FT-IR (ATR) spectra of pure PA (a) and NE (b).

(Table 4). Peaks at 1079 and 1579 were also detected in NE. The peak at 1079 indicates the presence of one cyanidin unit and one delphinidin unit as shown in Table 4. The peak at 2079 indicates the presence of two PC units and one PD unit. The presence of PD unit in NE was expected based on the ^{13}C NMR analysis. From MALDI TOF MS analysis, we con-

Table 4. Masses and unit types of Ac-NE by MALDI TOF MS

Polymer	M+Na ⁺ (positive reflectron)	Unit type	
		Cyanidin (<i>n</i>)	Delphinidin (<i>n'</i>)
Dimer	1021	2	0
	1079	1	1
	1037	0	0
Trimer	1519	3	0
	1579	2	1
	1637	1	2
Tetramer	2018	4	0
	2076	3	1
	2134	0	0
	2192	0	0
Pentamer	2515	5	0
	2574	4	1
	2632	0	0
	2690	0	0

Note: Calculated $[M+Na]^+ = M' + (n+n'-1)(M'-2H) + 58n' + 23$, M': 500 Da, H: 1 Da, Na⁺ adduct: 23 Da, -OAc group: 58 Da

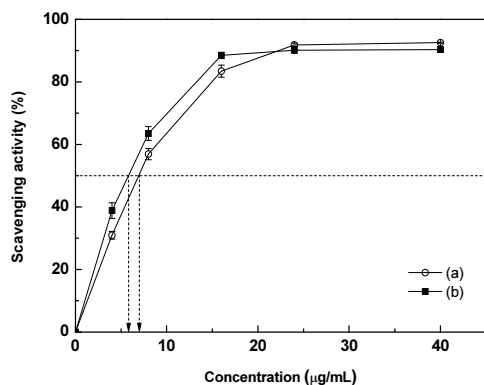


Fig. 6. DPPH free radical scavenging activities of pure PA (a) and NE (b).

cluded NE were mainly composed of low MW PAs of dimers and trimers. The peak at 686 only appeared in NE but a structure could not be assigned and further studies will be needed for this peak assignment.

From MALDI TOF MS analysis, it was confirmed that the majority of the compounds present in NE were PC with traces of PD. This result highly corresponded with the ^{13}C NMR result. In MALDI TOF MS analysis, flight time varies depending on the temperature and the voltage. Also, the laser intensity fluctuations affect the signal intensity of analytes, resulting in overlapping peaks of compounds of similar MW (Vorm *et al.*, 1994; He *et al.*, 1995). In fact, the MW of PCs in this experiment was very similar to that of PCs isolated by Ku and Mun (Ku and Mun, 2007).

3.3. Antioxidant activity

The antioxidant activity of NE was determined by DPPH free radical scavenging assay. Fig. 6 shows results for pure PA and NE. Both pure PA and NE showed an overall similar tendency in free radical scavenging activity, but it was thought that NE had slightly higher antioxidant activity than pure PA. Specifically, the concentration of DPPH free radical required to reach 50% scavenging activity was 5.9 $\mu\text{g}/\text{mL}$ for NE but 7 $\mu\text{g}/\text{mL}$ for pure PA. We speculated that NE exhibited a slightly higher activity than pure PA due to the presence of a component having a higher antioxidant activity in addition to the main PA component present in NE. This assay confirmed that NE, like pure PA, exhibited strong antioxidant activity even at extremely low concentrations.

4. CONCLUSION

When *P. radiata* bark was extracted with 0.8% NaHCO_3 solution, the neutral extract (NE) with a pH of 6.66 was obtained in 44% yield, which accounts

for about 60% of the 1% NaOH extract. The NE was characterized through various instrumental analyses. GPC analysis showed that NE had a wider MW distribution and lower \bar{M}_w (5,300 Da) than pure PA. From the FT-IR, ^{13}C NMR, and MALDI TOF MS analyses, it was confirmed that NE was mainly composed of PAs consisting of PC units and small amount of sugars as a minor component. NE exhibited a potent antioxidant activity similar to pure PA. Consequently, the weak alkaline extraction of *P. radiata* bark not only had a high extract yield, but also had high antioxidant activity and solubility in cold water, so the extract would be beneficial to the health supplement, functional cosmetic, and natural dyestuff industries in the future.

ACKNOWLEDGMENT

Authors would like to acknowledge Ms. Ji Young Oh (CURF, Jeonbuk National University) and Dr. Sae Jin Lee (Korea Basic Science Institute, Jeonju) for conducting ^{13}C NMR and MALDI TOF MS analysis, respectively.

REFERENCES

- Bayne, K. 2015. Wood quality considerations for radiata pine in international markets. *New Zealand Journal of Forestry* 59(4): 23-31.
- Choi, Y.H., Song, C.H., Mun, S.P. 2018. Proanthocyanidin-rich *Pinus radiata* bark extract inhibits mast cell-mediated anaphylaxis-like reactions. *Phytotherapy Research* 32(2): 290-297.
- Ghosh, D., Mukherjee, P.K. 2009. *Natural Medicines: Clinical Efficacy, Safety and Quality*. 1st Ed, CRC Press, Florida, USA.
- He, L., Liang, L., Lubman, D.M. 1995. Continuous-flow MALDI mass spectrometry using an ion trap/reflectron time-of-flight detector. *Analytical Chemistry* 67(22): 4127-4132.
- Jeong, S.H., Mun, S.P. 2018. Preparation and chemical characterization of alkaline extracts from *Pinus radiata* bark. Jinju, Republic of Korea, Proc. of 2018 the Korean Society of Wood Science and Technology (KSWST 2005), p. 101.
- Kim, Y.G., Park, H.Y. 2004. The effects of Pycnogenol on DNA damage in vitro and expression of superoxide dismutase and HP1 in *Escherichia coli* SOD and catalase deficient mutant cells. *Phytotherapy Research* 18(11): 900-905.
- Korea Forest Service (KFS). 2020. *Statistical Yearbook of Forestry* (Report No. 49).
- Kraus, T.E., Yu, Z., Preston, C.M., Dahlgren, R.A., Zasoski, R.J. 2003. Linking chemical reactivity and protein precipitation to structural characteristics of foliar tannins. *Journal of Chemical Ecology* 29(3): 703-730.
- Ku, C.S., Mun S.P. 2008. Antioxidant properties of monomeric, oligomeric, and polymeric fractions in hot water extract from *Pinus radiata* bark. *Wood Science and Technology* 42(1): 47-60.
- Ku, C.S., Mun, S.P. 2007. Characterization of proanthocyanidin in hot water extract isolate from *Pinus radiata* bark. *Wood Science and Technology* 41(3): 235-247.
- Ku, C.S., Mun, S.P., Jang, J.P. 2011. Effects of water extraction temperatures on the yield, molecular weight, and antioxidant activity of proanthocyanidins extracted from *Pinus radiata* bark. *Forest Products Journal* 61(4): 321-325.
- Mun, S.P. 2014. Efficacy and reusability of commercial adsorbent for isolation of proanthocyanidins from hot water extract of *Pinus radiata* bark. *Journal of the Korean Wood Science and Technology* 42(2): 207-213.
- Mun, S.P., Lim, S.Y. 2008. Preparation of proanthocyanidin-rich extract from *Pinus radiata* bark by weak-alkaline treatment. Jinju, Republic

- of Korea, Proc. of 2008 the Korean Forestry Society (KFS), pp. 410-413.
- Mun, S.P., Nicholas, D. D. 2017. Effect of proanthocyanidin-rich extracts from *Pinus radiata* bark on termite feeding deterrence. Journal of the Korean Wood Science and Technology 45(6): 720-727.
- Murphy, G., Cown, D. 2015. Within-tree, between-tree, and geospatial variation in estimated *Pinus radiata* bark volume and weight in New Zealand. New Zealand Journal of Forestry Science, 45(1): article No. 18, 1-7.
- Newman, R.H., Porter, L.J. 1992. Solid state ¹³C-NMR studies on condensed tannins. In: Plant polyphenols, Basic Life Science 59, Ed. by Hemingway, R.W., Laks, P.E., Springer, Boston, USA, p 346.
- Packer, L., Rimbach, G., Virgili, F. 1999. Antioxidant activity and biologic properties of a proanthocyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. Free Radical Biology and Medicine 27(5-6): 704-724.
- Pavia, D.L., Lampman, G.M., Kriz, G.S. 1996. Introduction to spectroscopy: A guide for students of organic chemistry. 2nd Ed, Harcourt Brace College Publishers, California, USA, pp. 49-50.
- Pizzi, A. 1982. Pine tannin adhesives for particleboard. Holz als Roh- und Werkstoff 40: 293-301.
- Prior, R.L., Gu, L. 2005. Occurrence and biological significance of proanthocyanidins in the American diet. Phytochemistry 66(18): 2264-2280.
- Rohdewald, P. 2002. A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. International Journal of Clinical Pharmacology and Therapeutics 40(4): 158-168.
- Sealy-Fisher, V.J., Pizzi, A. 1992. Increased pine tannins extraction and wood adhesives development by phlobaphenes minimization. Holz als Roh- und Werkstoff 50(5): 212-220.
- TAPPI (Technical Association of the Pulp and Paper Industry) Method. Solvent extractives of wood and pulp, T 204 cm-07. 2007.
- TAPPI (Technical Association of the Pulp and Paper Industry) Method. Water solubility of wood and pulp, T 207 cm-08. 2008.
- TAPPI (Technical Association of the Pulp and Paper Industry) Method. Ash in wood, pulp, paper and paperboard: combustion at 525°C, T 211 om-02. 2002.
- TAPPI (Technical Association of the Pulp and Paper Industry) Method. One percent sodium hydroxide solubility of wood and pulp, T 212 om-02. 2002.
- TAPPI (Technical Association of the Pulp and Paper Industry) Method. Acid-insoluble lignin in wood and pulp, T 222 om-06. 2006.
- Vorm, O., Roepstorff, P., Mann, M. 1994. Improved resolution and very high sensitivity in MALDI TOF of matrix surfaces made by fast evaporation. Analytical Chemistry 66(19): 3281-3287.
- Yazaki, Y., Hillis, W. 1977. Polyphenolic extractives of *Pinus radiata* bark. Holzforschung 31(1): 20-25.