Proanthocyanidin-rich Extract from *Pinus radiata* Bark: Mild-Alkaline Extraction and Characterization

Sung Phil Mun

This study assessed the efficacy of mild-alkaline extraction from *P. radiata* bark in obtaining proanthocyanidin (PA)-rich extracts. When the bark was treated with three types of bases—Na_2CO_3, $NaHCO_3$, and NaOH—at varying concentrations, the extract yields increased with higher concentrations. When the pH of the extracts exceeded 7, the PA content and antioxidant activity were remarkably reduced. This result suggests that the pH holds a greater effect in the alkaline extraction of the bark rather than the type of base used. Among the bases used, $NaHCO_3$ was selected and the extraction conditions of pine bark were examined at a concentration where the pH of the extract did not exceed 7. The extraction time during mild-alkaline extraction using 0.2% $NaHCO_3$ was reduced compared to water-only extraction at the same temperature. Moreover, the extract yields were over 10% higher than those of water extraction, and the dried extracts exhibited good solubility in water. The mild-alkaline extracts were characterized using FT-IR and ^{13}C NMR spectroscopic techniques, and acidic alcoholysis. Analyses of the spectra of the mild-alkaline extracts showed similarities to that of pure PA and hot water extract. This result indicated that PA in the bark was not significantly affected during mild-alkaline extraction.

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Keywords: Pinus radiata bark; Proanthocyanidin (PA)-rich extracts; Mild-alkaline extraction; PA content; Antioxidant activity

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INTRODUCTION

Proanthocyanidins (PA, Fig. 1) are a class of polyphenols known for their potent antioxidant properties, abundantly found in fruits, peels, and tree barks (Smeriglio *et al.* 2017). PA belongs to the group of condensed tannins, which are already extensively employed in leather industry and as natural dyes (Song *et al.* 2009; Mun *et al.* 2021; Brudzyńska *et al.* 2022). Moreover, Pycnogenol®, Enzogenol®, and Pinoradiol®, derived from the bark of *Pinus maritima* or *Pinus radiata*, are majorly composed of PA, and are widely used as key ingredients in health supplements and functional cosmetics (Packer *et al.* 1999; Rohdewald 2002; Shand *et al.* 2003; Mun 2009; Fravel *et al.* 2012; Kim *et al.* 2016; Thamizhiniyan *et al.* 2016).

Pinus radiata, commonly known as radiata pine, is extensively planted in New Zealand, Chile, and Australia. South Korea stands out as one of the world's major importers of *P. radiata* wood, accounting for 85% of the country's softwood imports (KFS 2019). Traditionally, the bark of pine trees is removed before utilizing the wood, and the discarded bark is primarily used as fuel or as soil amendment. However, it is worth noting that *P. radiata* bark contains a considerable amount of polyphenols, with 50 to 60% of its

composition comprising these compounds, and among them, 70 to 80% are composed of valuable PA (Lee *et al.* 2020). The methods for extracting PA from *P. radiata* bark have been investigated by Ku and Mun (2007) and Mun (2014). Extraction of PA from the bark is primarily achieved through hot water extraction, but only about one-third of the total bark PA can be extracted using this method (Lee *et al.* 2020). In addition, the hot water extract (HWE) after drying has poor solubility in water, which makes further utilization difficult. On the other hand, pine bark polyphenols are highly soluble in alkali, and currently they are mostly extracted using 1% NaOH. However, during alkali extraction using strong bases, PA, the main component of pine bark, undergoes severe structural changes, leading to the formation of insoluble phlobaphenes (Fig. 1, Young *et al.* 1985; Steynberg *et al.* 1986; Sealy-Fisher and Pizzi 1992). The latter exhibit very low antioxidant activity. In other words, alkaline extraction facilitates mass extraction of PA, but it does not preserve the inherent antioxidant activity of PA. However, if the conditions can be established to enable efficient extraction of PA from the bark while minimizing their structural changes during alkaline extraction and simultaneously preserving the potent antioxidant activity of PA, then alkaline extraction could become an appealing method. Therefore, this study initially examined the extraction conditions using various concentrations of bases ($NaHCO_3$, Na_2CO_3, and NaOH) from *P. radiata* bark to achieve both high PA yield and potent antioxidant activity. The structural changes that occurred in PA, obtained the alkaline extraction, were also investigated using spectroscopic techniques.

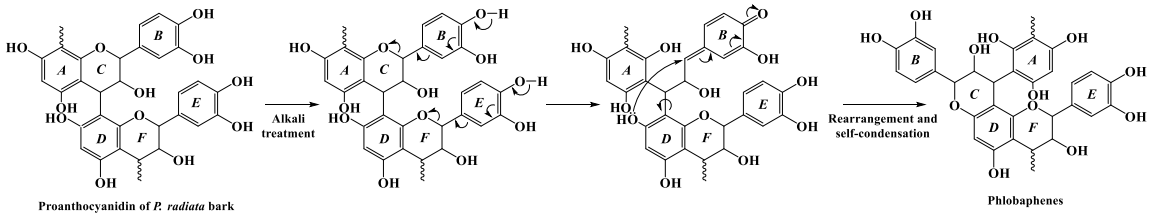

Fig. 1. Mechanism of rearrangement and self-condensation of proanthocyanidins to phlobaphenes. The mechanism of phlobaphene formation was based on the structure proposed by Young *et al.* (1985) and Steynberg *et al.* (1986), with minor modifications.

EXPERIMENTAL

Materials

P. radiata bark was kindly provided by Hanyoung Wood Co., Ltd. (Gunsan, Korea). The bark was air-dried at room temperature for 3 days, then dried further in a convection oven at 60±1 °C for 48 h. After removing the outer bark scales and inner bark, the dried bark was then pulverized using a high-speed mill equipped with a 1 mm screen. The bark powder was stored in a zipper bag at 4 °C until further use.

Sodium carbonate (Na_2CO_3, 99.5%), sodium bicarbonate ($NaHCO_3$, 99%), sodium hydroxide (NaOH, 93%), and concentrated HCl (35%) were purchased from Duksan Pure Chemicals (Ansan, Korea). 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was purchased from Sigma-Aldrich (St. Louis, USA). D_2O and acetone-d_6 for ^{13}C NMR analysis were purchased from Eurisotop (Saint-Aubin, France).

Methods

Chemical composition of P. radiata bark

The chemical composition of *P. radiata* bark, *i.e.* ash content, cold water extract, hot water extract, 1% NaOH extract, and total lignin content, was determined in accordance with TAPPI test methods. The acid-soluble lignin content was measured at a wavelength of 204 nm with an absorption coefficient of 105 L/g·cm. When quantifying the lignin content in pine bark using a strong acid like 72% H_2SO_4, the value is typically overestimated. This is due to the high content of polyphenols in the bark, which precipitates along with lignin during strong acid treatment. For this reason, the polyphenols in the bark were first removed by 1% NaOH extraction. Subsequently, the polyphenol-corrected lignin content was determined using the residues remaining after the 1% NaOH extraction. The polyphenol content was obtained from the difference between Klason lignin values before and after 1% NaOH extraction.

Alkaline extraction

A 2 g (oven dry weight, o.d.) of *P. radiata* bark powder (1 mm pass) was subjected to alkaline extraction using three different bases such as NaOH, $NaHCO_3$, and Na_2CO_3 with varying concentrations. The extraction temperature, liquor-to-bark ratio, and time were fixed at 80 °C, 10:1, and 5 min, respectively. The resulting mixture after each extraction was filtered using a 1G3 glass filter, and the residue was thoroughly washed with distilled-deionized (DDI) water, dried overnight at 105 °C to determine the extract yield. The extracts and washings were combined and subjected to lyophilization. The lyophilized sample was dried in a vacuum oven in the presence of P_2O_5 prior to further analysis.

To determine the optimal conditions for alkaline extraction of pine bark, $NaHCO_3$ was selected from the three types of bases used in this study, and its concentration was fixed at 0.2%. Extraction variables including temperature (20 to 100 °C), time (5 to 60 min), and liquor-to-bark ratio (5 to 50) were investigated. The amount of sample and post-alkaline extraction processes was carried out as described above. All extractions were conducted in duplicate, and the average values were shown.

Antioxidant activity

The antioxidant activity of the extracts was determined using DPPH radical scavenging assay. To prepare the stock solution, 10 mg of the extract was dissolved in 10 mL of MeOH, and the sample solution was prepared by diluting 1 mL of the stock solution with MeOH. Then, 100 µL of the sample solution and 200 µL of 0.1 mM DPPH solution were mixed, and the mixture was incubated at 25 °C for 30 min. The absorbance of the resulting solution was measured at 515 nm using a Microplate Reader (ELx-808TM, Bio Tek Instruments, Inc., Winooski, USA). The assay was performed in triplicate. The DPPH radical scavenging activity was calculated according to Eq. 1,

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

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

Quantification and monomeric composition of PA

The *n*-BuOH–HCl assay was conducted according to the method outlined by Hagerman (2002) for determining the PA content and monomeric composition of PA in

the extracts. One milligram of extract was placed into a PTFE screw-capped vial and dissolved in 1 mL of 50% (v/v) aqueous MeOH. Subsequently, 6.0 mL of *n*-BuOH–HCl reagent (95:5, v/v) and 0.2 mL of iron reagent (2% $FeNH_4(SO_4)_2$ in 2 N HCl) were added into the vial. The mixture was heated to 95 to 100 °C for 1 h. After the reaction, a visible spectrum in the range of 400 to 700 nm was plotted using a UV/Vis spectrophotometer (Hewlett Packard 8452A, Palo Alto, USA) to determine the monomeric composition of PA in the extracts. Calculation for PA content in the extracts was based on a calibration curve obtained by pure PA previously prepared from the same pine bark (Ku and Mun 2007). The assay was performed in duplicate. The PA content was expressed as percentage of pure PA equivalents.

FT-IR spectroscopy

Briefly, 1 mg of extract prepared under different conditions was mixed with 100 mg of KBr, then ground and compressed to make thin discs. The IR spectra of the prepared sample discs were recorded in the transmission mode using an FT-IR spectrophotometer (Shimadzu 8201 PC, Kyoto, Japan).

^{13}C NMR analysis

About 100 mg of each extract prepared from pine bark, 0.4 mL of D_2O, and 0.2 mL of acetone-d_6 were added into a 5 mL conical beaker. The beaker was sonicated for 1 min to completely dissolve the sample. The solution was filtered, and the filtrate was immediately transferred into an NMR tube. The conical beaker was washed with a mixture of D_2O and acetone-d_6 in a 2:1 (v/v) ratio, and then filtered on the same filter. All solutions were received into the NMR tube again. The measurement was conducted with a 400 MHz FT-NMR spectrometer (AL-400, JEOL, Tokyo, Japan) in the Center for University-wide Research Facilities (CURF) at Jeonbuk National University.

RESULTS AND DISCUSSION

Chemical Composition of *P. radiata* Bark

The barks of *Pinus* species contain abundant polyphenols and extractives (Ku *et al.* 2007). The *P. radiata* bark used in this study also exhibited high contents of cold water, hot water, and 1% NaOH extracts, as shown in Table 1. In particular, the 1% NaOH extract constituted as much as 64.8%, which indicates that extractives soluble in 1% NaOH are the major component of the bark. The Klason lignin content of the extractives-free *P. radiata* bark was 70.4%, which is exceptionally high. This unusually high lignin content arises from the fact that, due to the strong acid (72% H_2SO_4) treatment during Klason lignin determination, polyphenols become insoluble along with lignin and are thus measured together with lignin. Therefore, in order to obtain the actual lignin content, the polyphenols must be removed. After removing the majority of polyphenols from the bark with 1% NaOH extraction, the Klason lignin content markedly decreased from 70.4% to 16.4%. Thus, the difference between Klason lignin content before and after 1% NaOH extraction can be attributed to the presence of polyphenols in the bark. In addition, the difference in Klason lignin values before and after 1% NaOH extraction can be assumed as the total polyphenol content. Based on this calculation, the total polyphenol content in the bark was 54.0%, which indicates that 83% of the 1% NaOH extract is composed of polyphenols.

Table 1. Chemical Composition of *P. radiata* Bark

Ash (%)	0.4
Extracts (%)	
Cold water	10.6
Hot water	23.4
1% NaOH	64.8
Alcohol-benzene	10.5
Lignin (%, based on bark) before 1% NaOH extraction	
Klason[1]	70.4
Acid soluble	9.6
Total	80.0
Lignin (%, based on bark) after 1% NaOH extraction	
Klason[2]	16.4
Acid soluble	0.2
Total	16.6
Total polyphenol (%) = 1 – 2	54.0

Alkaline Extraction

Effect of alkali type and concentration on extract pH and yield, PA content, and antioxidant activity

The alkaline extraction of *P. radiata* bark was performed using three different bases, *i.e.*, Na_2CO_3, $NaHCO_3$, and NaOH, with varying concentrations (0.095 to 1.000%). The extraction was based on conditions that exhibited the highest antioxidant activity in a previous study on the extraction of the same pine bark (Ku *et al.* 2011). Thus, the extraction was performed at a temperature of 80 °C, an extraction time of 5 min, and a liquid-to-bark ratio of 10:1. Table 2 shows the results of pH of filtrate, extract yield, PA content, and antioxidant activity after alkaline extraction. The extract yield increased as the alkali concentration increased, regardless of the type of alkali used. The extract yield obtained by alkaline extraction of the bark was higher compared to hot water extraction, which was carried out as a control. These results were attributed to the alkaline-induced neutralization of PA, the main constituent in the bark, leading to an increase in their solubility, thereby facilitating their extraction from the bark. The PA content was determined using the *n*-BuOH–HCl assay, with pure PA isolated and purified from the same pine bark used as the standard material. However, PA content in the extracts, including those from HWE, and extracts with a pH of 7 or below, showed values exceeding 100%. The *n*-BuOH–HCl assay is a widely employed method for determining condensed tannins or PA in plant extracts, but variations in the structural composition of PA from different sources can lead to differing reactivity in this assay. In addition, PA with higher degrees of polymerization yield more anthocyanidins than smaller compounds, such as dimers. Notably, prodelphinidin (PD) units have been observed to produce higher amounts of anthocyanidins compared to procyanidin (PC) units (Wallace and Giusti 2010; Shay *et al.* 2017). Consequently, some results in PA content particularly those obtained at pH 7 or lower may reflect overestimated values. While this method may have challenges in accurately quantifying PA as mentioned earlier, it still enables a relative comparison between treatments. In Table 2, when the alkali concentration is increased and the pH of the extracts reached 7 or higher, the PA content and antioxidant activity remarkably decreased. Figure 2 illustrates the relationship between the pH of extracts obtained after different extraction of the bark and PA contents and DPPH radical scavenging activity. When the pH of the extracts exceeded 7, both the PA content and antioxidant activity

notably decreased. These results, as mentioned earlier, can be attributed to the structural modification (rearrangement and self-condensation) of PA into hydrophobic phlobaphenes under sufficiently harsh alkaline extraction conditions (Fig. 1). Therefore, maintaining the pH of the extracts by alkaline extraction of the bark below 7 is crucial. Figure 3 shows the relationship between PA content and DPPH radical scavenging activity. As anticipated from Fig. 2, there was almost a direct relationship between PA content and antioxidant activity. A high correlation coefficient was observed between the two variables. These results are consistent with a previous work demonstrating a linear correlation between PA content and antioxidant activity in hot water extracts from various pine barks (Ku *et al.* 2007).

Table 2. Results of Extract pH and Yield, PA content, and DPPH Radical Scavenging Activity

Extraction	Concentration (%, w/v)	Extract pH	Extract Yield (%)	PA (%)	DPPH Radical Scavenging Activity (%)
Water		3.41	18.9	130	80
$NaHCO_3$	**0.200**	**6.31**	**26.5**	**115**	**77**
	0.400	**6.93**	**29.7**	**116**	**80**
	0.800	7.76	30.5	50	43
	1.000	8.00	29.9	60	49
Na_2CO_3	**0.125**	**5.79**	**25.8**	**113**	**72**
	0.250	7.46	28.3	86	65
	0.500	8.14	30.2	37	35
	1.000	9.33	33.7	14	14
NaOH	**0.095**	**6.28**	**26.3**	**119**	**75**
	0.190	**7.75**	**31.0**	**110**	**79**
	1.000	10.06	40.2	19	22
Water extraction: liquor-to-bark ratio 10:1, 100 °C, 60 min; Alkaline extraction: liquor-to-bark ratio 10:1, 80 ℃, 5 min; PA content was determined by *n*-BuOH–HCl method.					

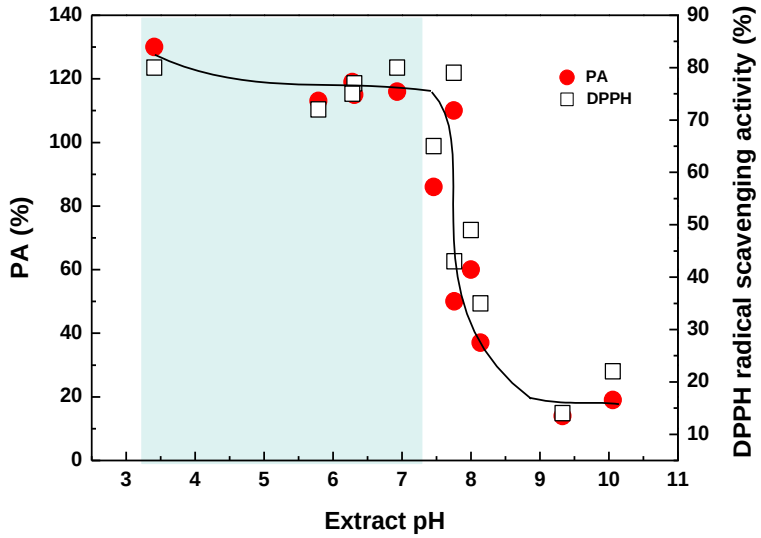

Fig. 2. Changes in PA content and DPPH radical scavenging activity on the extract pH

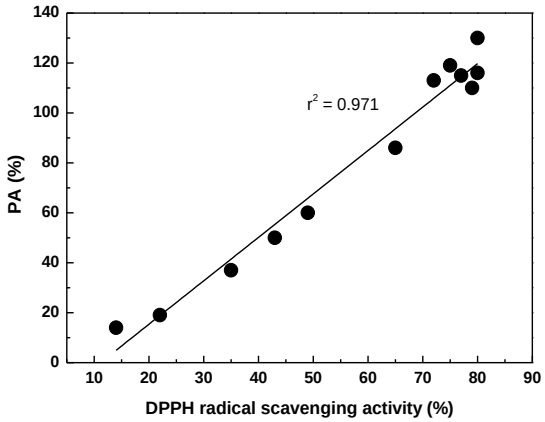

Fig. 3. Relationship between PA content and DPPH radical scavenging activity

Effect of temperature, time, and liquor ratio at 0.2% $NaHCO_3$ extraction conditions

When the pH of the extracts obtained through mild-alkaline extraction of pine bark was below 7, a higher yield of PA-rich extracts with high antioxidant activities could be obtained compared to hot water extraction method. Therefore, to establish optimal alkaline extraction conditions for the bark, the influence of extraction temperature, extraction time, and liquor-to-bark ratio at fixed alkaline concentrations were investigated. Among the three bases, $NaHCO_3$ was used in this experiment due to its mild alkalinity and ease of handling. The concentration was fixed at 0.2% because the pH of the extract was maintained below 7 at this concentration.

As shown in Fig. 4, when the extraction temperature was raised from room temperature to 100 °C, the extract yield increased from 19% to 32%. The use of 0.2% $NaHCO_3$ instead of water resulted in a low extraction temperature (20 to 40 °C) and a short extraction time of 5 min, achieving an extract yield similar or higher to that obtained by hot water extraction at 80 to 100 °C for 1 h in the figure shown for comparison purposes. Even at an extraction temperature of 40 °C, the extract yield after 0.2% $NaHCO_3$ extraction was more than 4% higher than that obtained using pressurized water at 120 °C. This means that the addition of a small amount of mild alkali can efficiently extract large amounts of bioactive extracts from *P. radiata* bark in a short period of time.

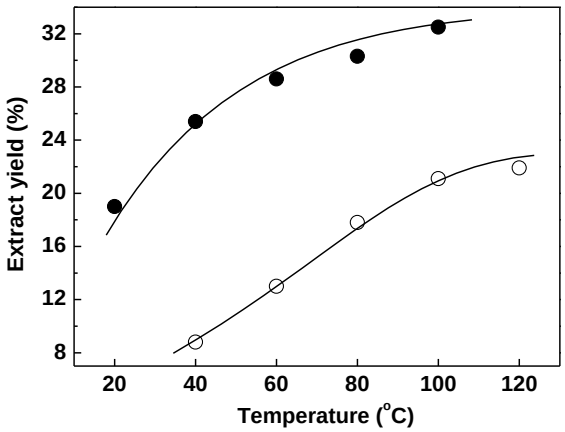

Fig. 4. Effect of extraction temperature on extract yield in 0.2% $NaHCO_3$ (●) and water (○) extraction. 0.2% $NaHCO_3$ extraction: liquor-to-bark ratio 10:1, extraction time: 5 min at each temperature; Water extraction: liquor-to-bark ratio 10:1, extraction time: 1 h at each temperature

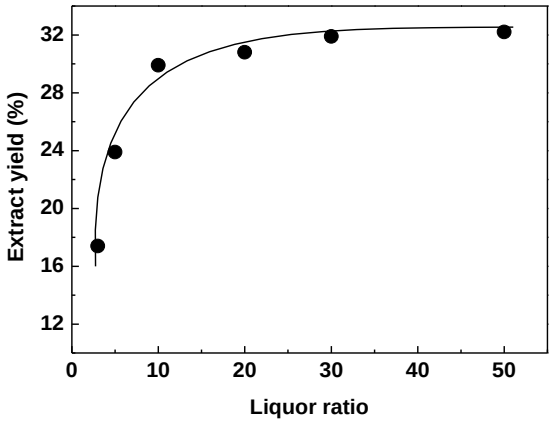

Fig. 5. Effect of liquor ratio on extract yield. Extraction temperature: 100 °C, extraction time: 5 min

The liquor-to-bark ratio is also one of the important factors that must be considered for commercial production of PA-rich extracts. Figure 5 represents the effect of liquor ratio on the extract yield in 0.2% $NaHCO_3$ extraction. The extract yield increased dramatically with an increase in liquor ratio. At a liquor ratio of 10, the extract yield was about 30%, but then increasing the liquor ratio to 50 only increased the yield of the extract by 2 to 3%. This indicates that the liquor ratio is an important variable in 0.2% $NaHCO_3$ extraction, but liquor ratios higher than 10 are unnecessary. Moreover, a high liquor ratio is not economically advantageous because it requires a considerable amount of energy in the concentration process to produce powder extracts.

Figure 6 depicts the variation in extraction yield and pH with respect to extraction time at a liquor-to-bark ratio 10:1 and an extraction temperature of 80 °C. At an extraction time of 10 min, the extract yield already reached 31%, and extending the time did not result in an increase in yield. This indicates that under mild-alkaline extraction conditions, a substantial production of active extracts can be achieved in a short period of time. The pH of the extract also followed an analogous trend to the extraction yield, and it was almost constant at pH 6 after 10 min of extraction. Based on these results, an extraction time of 5 to 10 min was considered suitable. In addition, mild-alkaline extracts dissolve well in water, unlike hot water extract (HWE).

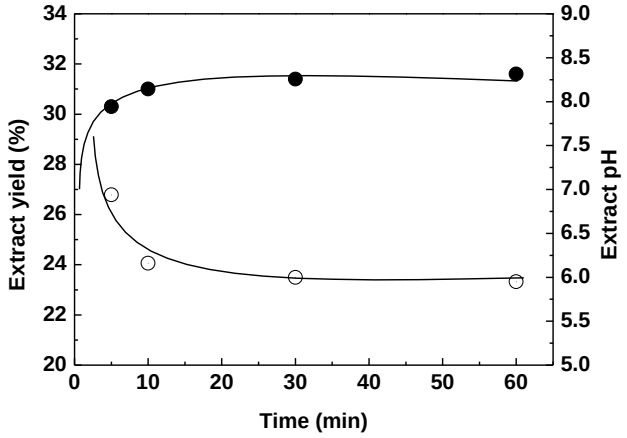

Fig. 6. Effect of extraction time on extract yield (●) and pH (○). Extraction temperature: 80 °C, liquor-to-bark ratio 10:1

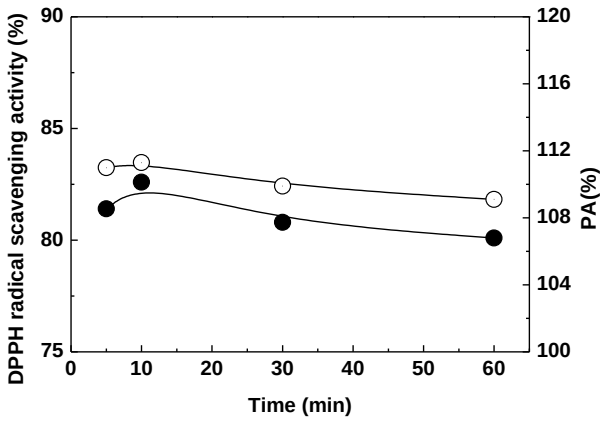

Fig. 7. Effect of extraction time on DPPH radical scavenging activity (○) and PA content (●). Extraction conditions are the same as Fig. 6.

Figure 7 shows the effect of extraction time on DPPH free radical scavenging activity and PA content under conditions of 80 °C and a liquor ratio of 10. The DPPH free radical scavenging activity and PA content were highest at an extraction time of 10 min and slightly decreased thereafter. However, these differences were almost negligible.

Based on these results, the important factors in the alkaline extraction of *P. radiata* bark are extraction temperature, extraction time, and liquid-to-bark ratio. In addition, to obtain an extract with high antioxidant activity and PA content from the bark of radiata pine using mild alkali, the pH of the extract should not exceed 7.

Characteristics of Alkaline Extracts

FT-IR, ^{13}C NMR spectroscopy, and alcoholysis were conducted to investigate the chemical composition and structural changes in the extracts obtained through mild-alkaline treatment of pine bark. FT-IR spectroscopy provides useful information regarding functional groups and their relative amounts. Figure 8 shows the IR spectra of pure PA, HWE, 0.2% $NaHCO_3$, and 1.0% NaOH extracts. The IR spectra of HWE and 0.2% $NaHCO_3$ extract were similar to that of pure PA. This outcome suggests that mild-alkaline extraction using $NaHCO_3$ extraction does not affect the main component, PA, in *P. radiata* bark. As shown in the IR spectra of pure PA, HWE, and 0.2% $NaHCO_3$ extracts, strong absorbance bands observed at 1520 cm^{-1} and 779 cm^{-1} are known to originate from the aromatic ring breathing mode and C–H out-of-plane deformation with two adjacent free hydrogen atoms, respectively (Foo 1981). These findings indicate the presence of the procyanidin (PC) structure (Foo 1981). On the other hand, the absorbance band of 1% NaOH extract was broader than that of pure PA, HWE and 0.2% $NaHCO_3$ extract. Especially, the absorbance bands at 1605, 1520, and 1440 cm^{-1}, which correspond to specific absorbance bands of the aromatic rings constituting PA, were much broader. These results indicate that structural changes occurred in the aromatic rings of PA due to strong alkaline extraction.

^{13}C NMR spectroscopy was employed to obtain a more detailed information on the chemical structures and composition of the alkaline extracts. The assignments of the NMR signals were based on the publications of Czochanska *et al.* (1980). Figure 9 shows the ^{13}C NMR spectra of pure PA, HWE, 0.2% $NaHCO_3$, and 1% NaOH extracts. The ^{13}C NMR spectrum of 0.2% $NaHCO_3$ extract was similar to that of HWE. In addition, most of the

peaks appearing in the ^{13}C NMR spectrum of 0.2% $NaHCO_3$ extract were also present in pure PA. This result suggests that mild-alkaline extraction of pine bark can not only produce better yields of PA-rich extract compared to conventional hot water extraction, but also reduce structural changes in the basic units that make up PA. In the ^{13}C NMR spectrum of the extract obtained using 1% NaOH, most peaks within the range of 110 ppm to 60 ppm had almost disappeared. The disappearance of these peaks is thought to be due to the formation of phlobaphenes by cleavage, followed by rearrangement of the C ring within the basic unit of PA. As evidenced by the ^{13}C NMR results, the use of strong alkali during pine bark extraction gave rise to severe structural changes in PA. The peaks at 145, 120, and 116 ppm are attributed to procyanidin (PC) units, while the peaks at 107 ppm correspond to prodelphinidin (PD) units in PA. The PC/PD ratio of PA can be determined by the intensity ratio of 116/107 ppm in the ^{13}C NMR spectrum (Kraus *et al.* 2003). For 0.2% $NaHCO_3$ extract, the intensity of 116 ppm was stronger than 107 ppm, indicating that most of the basic units constituting PA were PCs, which is consistent with the FT-IR analysis described above. Based on these results, it was evident that the 0.2% $NaHCO_3$ extract primarily consisted of PC units, similar to pure PA and HWE. In addition, several strong peaks in the range of 80 to 60 ppm observed in HWE and 0.2% $NaHCO_3$ extracts are attributed to carbohydrates. These carbohydrate-related peaks were scarcely detected in the 1% NaOH extract, likely due to the oxidative degradation of carbohydrates under hot and strong alkaline conditions.

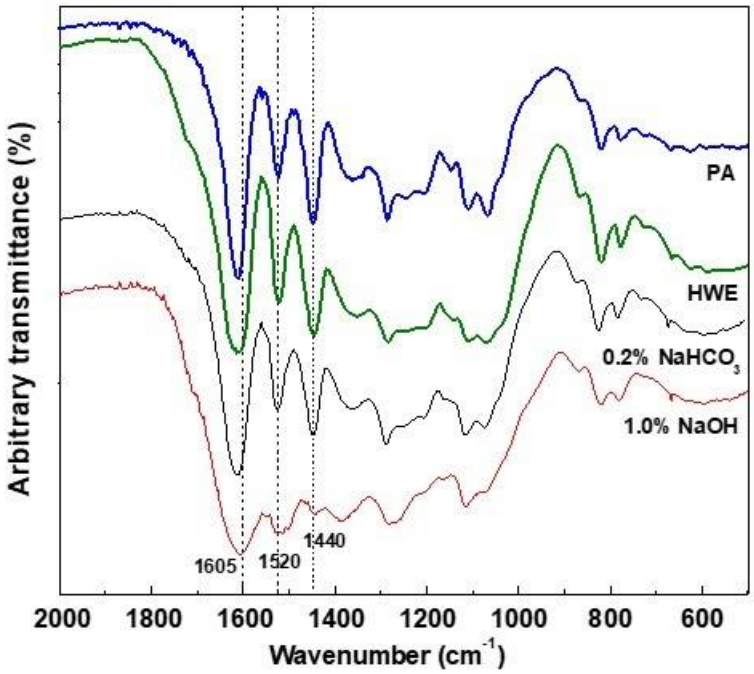

Fig. 8. FT-IR spectra of PA, HWE, 0.2% $NaHCO_3$, and 1% NaOH extracts

Figure 10 shows the visible spectra after acidic alcoholysis reaction of pure-PA, HWE, 0.2% $NaHCO_3$, and 1% NaOH extracts. In alcoholysis, using *n*-BuOH–HCl in the presence of Fe^{3+}, the interflavanoid bonds in PA are oxidatively cleaved by acidic alcohols (Hagerman 2002). The resulting dehydration product, red anthocyanins, can be estimated through colorimetric methods (Bate-Smith 1973). Samejima and Yoshimoto (1981) reported that (epi)catechin-based and gallo(epi)catechin-based PAs yield anthocyanidins

with λ_{max} values of 547 nm (cyanidin) and 558 nm (delphinidin), respectively. Hussein *et al.* (1990) suggested that variation of absorbance maxima in the *n*-BuOH–HCl reaction between 540 and 550 nm is due to the relative amounts of anthocyanidins (delphinidin to cyanidin ratio) formed during the alcoholysis. As shown in Fig. 10, the spectrum of 0.2% $NaHCO_3$ extract closely resembled that of pure PA, with the maximum absorbance peak residing at 546 nm, identical to pure PA.

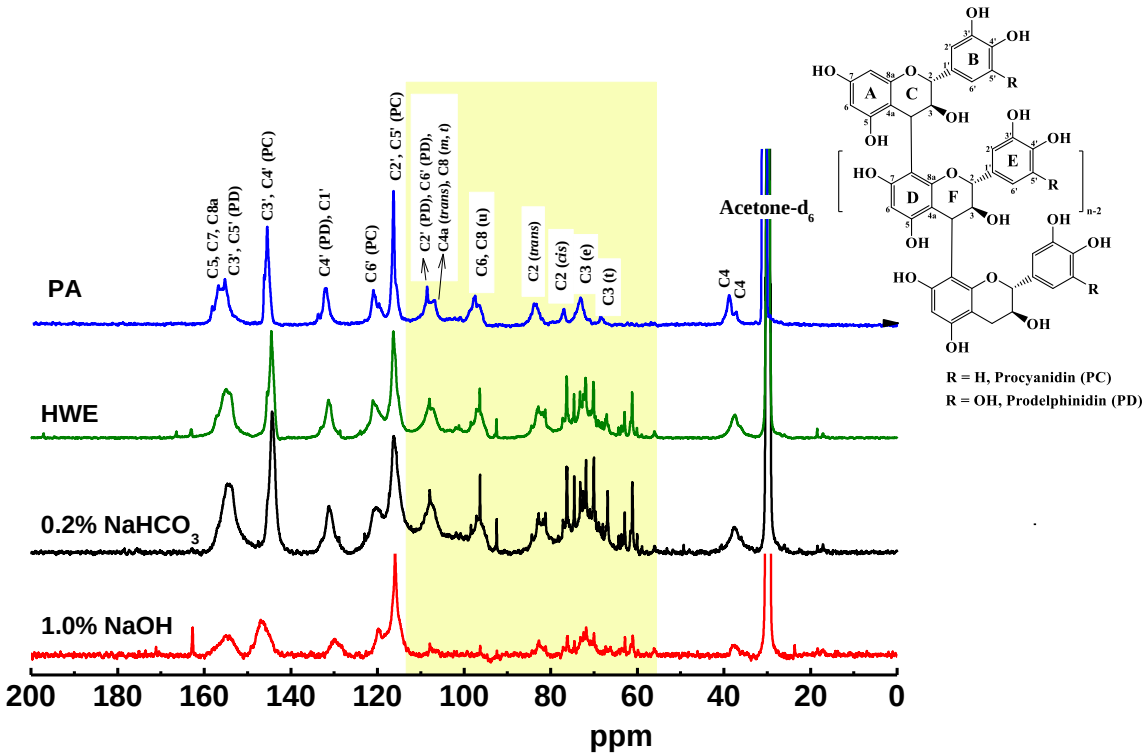

Fig. 9. ^{13}C NMR (400MHz) spectra of pure PA, HWE, 0.2% $NaHCO_3$, and 1.0% NaOH extracts in acetone-d_6/D_2O (1:2 v/v); t terminal unit, m middle unit, u upper unit, e external unit

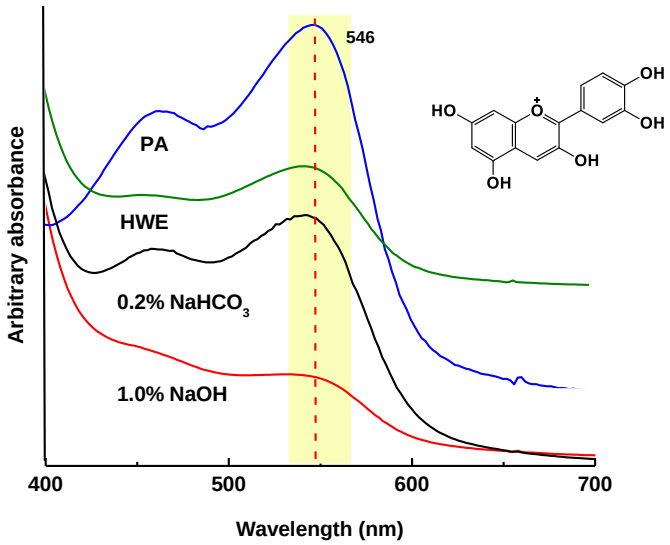

Fig. 10. Visible spectra of PA, HWE, 0.2% $NaHCO_3$, and 1% NaOH extracts after alcoholysis

Consequently, this suggests that PA in 0.2% $NaHCO_3$ extract is predominantly composed of cyanidins as the main extension unit, rather than delphinidins. On the other hand, the spectrum of the 1% NaOH extract differed from that of 0.2% $NaHCO_3$ extract, and the peak of the anthocyanidin originated from 500 nm to 600 nm almost disappeared and remained in the shoulder shape. This suggests that remarkable structural changes in PA occurred during 1% NaOH extraction, as indicated by FT-IR and ^{13}C NMR analyses.

CONCLUSIONS

1. Mild-alkaline extraction was performed to obtain water-soluble proanthocyanidin (PA)-rich extracts with high antioxidant activity from *P. radiata* bark. To achieve this objective, maintaining the pH of the extracts below 7 was crucial, regardless of the type of base used.
2. Mild-alkaline extraction resulted in an improved extract yield of over 10% compared to simple water or hot water extraction, and it allowed reduced extraction time. These results were attributed to the fact that PA, the main component of the bark, was neutralized by mild-alkali treatment, increasing its solubility in water and minimizing structural changes in PA by maintaining the pH below 7.
3. Mild-alkaline extraction is anticipated to significantly contribute to the advancement of related industries by enabling rapid mass production of high-value extracts from *P. radiata* bark in a relatively short period of time.

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