EFFICIENT EXTRACTION OF POLYPHENOLICS FROM THE BARK OF TROPICAL TREE SPECIES

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MAKINO R, OHARA S & HASHIDA K. 2009. Efficient extraction of polyphenolics from the bark of tropical tree species. The objective of this study was to discover a method for extracting polyphenolics containing as few sugar components as possible. In this work, water extraction was carried out for the bark of several tropical tree species under various temperature conditions to compare the extractability of polyphenolics and sugar components. The yield and total polyphenolics content of the extracts increased with increase in extraction temperature. Total polyphenolics at 100 ˚C from *Acacia mangium, Acacia auriculiformis, Rhizophora apiculata* and *Larix leptolepis* were 14.2, 12.9, 8.0 and 5.3% respectively. Total sugar contents from *A. mangium* and *L. leptolepis,* which were determined using a slightly modified phenol–sulphuric acid method, were almost constant at 20–80 ˚C, but increased substantially at 100 ˚C. The same trend was observed with total reducing sugar contents from these two species. Our results suggest that water extraction at 80 ˚C from the barks of *A. mangium* and *L. leptolepis* is a good method for the extraction of polyphenolics with very few sugar components.

Keywords: Water extraction, sugars, *Acacia mangium*, *Acacia auriculiformis*, *Rhizophora apiculata*, *Larix leptolepis*

MAKINO R, OHARA S & HASHIDA K. 2009. Pengekstrakan terbaik polifenol daripada kulit kayu spesies pokok tropika. Objektif kajian ini adalah untuk mencari kaedah mengekstrak polifenol yang mengandungi paling kurang komponen gula. Dalam penyiasatan ini, pengekstrakan berasaskan air dijalankan untuk beberapa kulit kayu spesies pokok tropika di bawah pelbagai suhu berbeza untuk membandingkan keterekstrakan polifenol dan komponen gula. Hasil dan jumlah kandungan polifenol ekstrak meningkat apabila suhu meningkat. Jumlah kandungan polifenol bagi kulit kayu *Acacia mangium, Acacia auriculiformis, Rhizophora apiculata* dan *Larix leptolepis* pada 100 ˚C adalah masing-masing 14.2%, 12.9%, 8.0% dan 5.3%. Jumlah gula daripada kulit kayu *A. mangium* dan *L. leptolepis* yang ditentukan dengan kaedah fenol–asid sulfurik terubah suai adalah hampir sama pada suhu 20 ˚C – 80 ˚C, tetapi meningkat dengan mendadak pada suhu 100 ˚C. Trend yang sama dicerap untuk kandungan gula penurun kedua-dua spesies ini. Keputusan kajian kami mencadangkan bahawa pengekstrakan berasaskan air menggunakan kulit kayu *A. mangium* and *L. leptolepis* pada suhu 80 ˚C merupakan kaedah terbaik bagi mengekstrak polifenol yang mengandungi sangat sedikit komponen gula.

INTRODUCTION

Large quantities of waste are generated by the wood industry, and it is an increasingly important task to develop methods for their effective utilization. The ratio of utilization of bark is considerably lower than that of other main wood waste types such as slab and sawdust. In Malaysia and Indonesia, some *Acacia* tree species are utilized as pulp chips and glued laminated timber. However, little of the bark generated at pulp mills and sawmills is utilized.

Condensed tannins are present at very high concentrations in the bark of timber species such as conifers (Aoyama *et al.* 1983), eucalypts (Cadahia *et al.* 1997) and leguminous trees (Ohara *et al.* 1994, Yazaki 1997). Many recent investigations on new utilizations of condensed tannins have been reported, focusing on their application in wood adhesives (Yazaki & Collins 1994), polyurethane foams (Ge & Sakai 1993) and as formaldehyde scavengers (Hashida *et al.* 2006). Our previous work (Ohara *et al.* 2004a) has demonstrated that when plywood was coated with a tannin-charcoal suspension prepared using 70% acetone aqueous extractives from *Acacia*

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mangium bark, the charcoal powder could be fixed on the surface of plywood after air-drying. We have also found that the presence of sugar components in the *A. mangium* extractives caused the elimination of charcoal powders on dropping of water (Ohara *et al.* 2004a). Therefore, it is useful to discover a method for extracting condensed tannins with the lowest possible presence of sugar components.

In this work, water extraction of the bark of the several tropical tree species under various temperature conditions was carried out to compare the extractability of condensed tannins and sugar components. The most efficient conditions for the extraction of tannins with the minimum possible presence of sugar components are discussed.

MATERIALS AND METHODS

Plant materials

Bark samples were collected from three tropical tree species and one popular Japanese tree species. *Acacia mangium* and *Acacia auriculiformis* were collected from Hanoi, Vietnam; the mangrove species *Rhizophora apiculata* from Serpong, Indonesia; and *Larix leptolepis* in Nagano Prefecture, Japan. These bark samples were used without separation of inner bark and outer bark. These samples were air-dried, ground in a Wiley mill and passed through a 0.5-mm screen.

Water extraction

Milled samples (0.5 g) obtained from *A. mangium*, *A. auriculiformis*, *R. apiculata* and *L. leptolepis* were extracted with 300 ml of water at 20 °C for two days. The extractives were then recovered by filtration through a 1G3 glass filter. The total amount of extractives was determined by weighing the residual sample on the glass filter. The filtrate was diluted with water until the total volume of the solution reached 500 ml. The aqueous solution obtained was used as the test solution for quantitative analyses of total polyphenolics, total sugars and total reducing sugars. The above milled samples (0.5 g) were also extracted with 100 ml of water at 40–100 °C in a reflux condenser for three hours and the extractives were recovered using the same procedure as above. The volume of filtrate was adjusted to 200 ml with water, and the aqueous solution obtained was also used as test solution for quantitative analyses.

Extraction with 70% acetone aqueous solution

Our pervious studies have shown that 70% acetone aqueous solution is a more efficient solvent for the extraction of polyphenols from tree bark than methanol or 80% methanol aqueous solution (Ohara *et al.* 1994, 2004b). Thus, in this study, the bark samples were extracted with 70% acetone aqueous solution to determine their polyphenol content. Milled samples (0.5 g) were extracted with 25 ml of 70% acetone aqueous solution at room temperature, and the extractives were recovered by centrifugation at 18 000 rpm for 20 min. The residual bark meal was re-extracted with 25 ml of 70% acetone aqueous solution. This procedure was repeated five times after which each supernatant was combined. The obtained solution was diluted with 70% acetone aqueous solution until the total volume was 200 ml. Total polyphenolics were then determined.

Total polyphenolics in extractives

Total polyphenolics in extractives were determined using a certain volume of each test solution according to the Folin-Ciocalteu method (Julkunen-Tiitto 1985). Folin-Ciocalteu phenol reagent was purchased from Kanto Kagaku Corporation, Tokyo, Japan. Total polyphenol content was shown as average per cent of oven-dried bark meal (w/w) and the experiment was carried out in duplicate. The calibration curve was determined using catechin as the standard sample.

Total sugars in extractives

Total sugars in extractives were measured according to the phenol-sulfuric acid method (Dubois *et al.* 1956) with a slight modification. Preliminary experiments have revealed that the presence of polyphenols such as flavanols and condensed tannins leads to overestimation of total sugar content when using the phenol-sulfuric acid method (Makino & Ohara 2001). Thus, in this study, determination of total sugars was done using a slightly modified version of the phenol-sulfuric acid method. A certain amount of each test solution was placed in a flask and evaporated to dryness. The residue was dissolved in 10 ml of water, transferred to a centrifuge tube, and then 10 ml of 1% lead acetate aqueous solution were added. After 20 min, the tube was centrifuged at 18 000 rpm for 20 min. To 2 ml of the supernatant transferred to a new centrifuge tube were added 0.05 ml of 80% phenol aqueous solution and 5 ml of concentrated sulfuric acid. After 35 min, the tube was centrifuged at 3500 rpm for 5 min, and the absorptivity of the supernatant was read at 490 nm. Total sugar content was also reported as average per cent of oven-dried bark meal (w/w) and the experiment was carried out in duplicate. The calibration curve was determined using glucose as the standard sample.

Total reducing sugars in extractives

Total reducing sugars in extractives were determined according to the Somogyi-Nelson method (Somogyi 1952, Nelson 1944). Alkaline-copper reagent and chromogenic reagent were prepared according to the above literature. As the quantitative analysis of reducing sugars according to the Somogyi-Nelson method was influenced by the presence of polyphenols, chromatography by Sephadex LH-20 column using water as an eluent was carried out to remove polyphenols from each test solution. A certain amount of test solution was applied to the Sephadex LH-20 column eluted with an appropriate amount of water. One milliliter of the eluent was pipetted into a narrow test-tube graduated at 25 ml. After addition of 1 ml of alkaline-copper reagent, the tube was covered with a stopper and then heated for 15 min in a boiling water bath. After termination of the reaction the tube was cooled in a pan of cold water. One milliliter of chromogenic reagent was added into the reaction products, and then the mixture was agitated and diluted to 25 ml with water. After 15 min the absorptivity of the mixture was measured at 660 nm. The experiment to determine total reducing sugar content was carried out in duplicate and the value was shown as average per cent of oven-dried bark meal (w/w). The calibration curve was determined using glucose as the standard sample.

RESULTS AND DISCUSSION

Extractability of polyphenolics by 70% acetone aqueous extraction

Results of the quantitative analyses are shown in Table 1 together with the extractive yields. Extractive yields from the bark of *A. mangium* and *A. auriculiformis* were 37.9 and 28.6%, and their polyphenol contents were 17.7 and 10.9% respectively. It has been reported that *A. mearnsii* bark, which is utilized as raw material in commercial wattle tannins, contains 38.0% polyphenols (Ohara *et al.* 1994). The present study showed that *A. mangium* and *A. auriculiformis* contained lower quantities of polyphenolics than *A. mearnsii*. In this study, *R. apiculata* contained 20.2% extractives and 8.0% polyphenolics. Polyphenolics in the bark of eight mangrove species grown in Thailand and Japan can be fully extracted by a two-step extraction method with methanol/distilled water (4/1, v/v) and then with distilled water alone and that the average polyphenol yield is 25.2% (Higaki *et al.* 1990), higher that the yield obtained for *R. apiculata* in the current study. *Larix leptolepis* contained 11.0% extractives and 4.6% polyphenolics; the latter lower than the 14.7% value reported by Samejima and Yoshimoto (1981) for the inner bark of *L. leptolepis*. Generally, the content of polyphenolics in inner bark of conifer is higher than that in its outer bark (Samejima & Yoshimoto 1982). In the present study, the content of polyphenolics was less than the result of Samejima and Yoshimoto (1982) probably because we used the whole bark. It appears that the polyphenol content varies depending on the ratio of inner bark to outer bark.

Table 1 Analytical data of 70% acetone aqueous extractives from the bark

Species	Extractives	Total polyphenolics
	(%)[1]	
Acacia mangium	37.9	17.7
Acacia auriculiformis	28.6	10.9
Rhizophora apiculata	20.2	8.0
Larix leptolepis	11.0	4.6

[1] % of oven-dried bark meal

Extractability of polyphenolics and sugars by water extraction

Results of quantitative analyses of polyphenolics and total sugars in water extractives are shown in Table 2 together with the extractive yields. The yield of extractives from all bark samples examined increased with increase in extraction temperature. Notably, the extractives yield from *L. leptolepis* largely depended upon the temperature, with the extractives yield at 100 ˚C being approximately threefold that at 20 ˚C. The extractives at 100 ˚C for *A. mangium, A. auriculiformis, R. apiculata* and *L. leptolepis* were 27.5, 25.0, 18.4 and 10.7% respectively. These yields are lower than those of the equivalent 70% acetone aqueous extractives.

Total polyphenolics content also increased when the temperature was higher, with values at 100 ˚C for *A. mangium, A. auriculiformis, R. apiculata* and *L. leptolepis* being 14.2, 12.9, 8.0 and 5.3% respectively. Generally, total polyphenolics contents from acetone and water extractives are quite close. These results indicate that water extraction at 100 ˚C is as effective as 70% acetone aqueous extraction for extracting polyphenolics from the bark.

As shown in Table 2, total sugar content was in the range of 0.4–2.6%. Although the total sugar content for *A. mangium* was almost the same at 20–80 ˚C, it increased substantially at 100 ˚C. This trend was also observed in *L. leptolepis*, but not in *A. auriculiformis* and *R. apiculata.*

All species showed similar total reducing sugar contents at all temperatures tested except for *A. mangium* and *L. leptolepis* which had increased values at 100 ˚C (Table 3). This tendency was similar to that of total sugar content. The total reducing sugar content was lower than the corresponding total sugar except in *R. apiculata.* It is, therefore, assumed that water extractives from *A. mangium, A. auriculiformis* and *L. leptolepis* contain mainly oligosaccharides and those from *R. apiculata* contain mainly monosaccharides, regardless of the extraction temperature. In terms of environment, water extraction is better than using organic solvent.

In this work, we found that the extractability of polyphenolics and sugar components from tree barks is different at various temperature

Table 2 Analytical data of water extractives from the bark

A. mangium

Water extraction temperature (°C)	Extractives	Total phenolics	Total sugars
		(%)[1]	
20	18.4	9.1	1.8
40	19.1	10.1	1.8
60	22.5	11.9	1.8
80	24.5	12.4	1.7
100	27.5	14.2	2.6

A. auriculiformis

Water extraction temperature (°C)	Extractives	Total phenolics	Total sugars
		(%)[1]	
20	18.9	8.9	1.3
40	18.6	9.6	1.4
60	21.5	11.3	1.6
80	23.0	11.7	1.5
100	25.0	12.9	1.7

R. apiculata

Water extraction temperature (°C)	Extractives	Total phenolics	Total sugars
		(%)[1]	
20	12.8	6.5	0.6
40	14.9	7.0	0.4
60	15.7	6.6	0.4
80	17.0	6.6	0.5
100	18.4	8.0	0.8

L. leptolepis

Water extraction temperature (°C)	Extractives	Total phenolics	Total sugars
		(%)[1]	
20	2.9	2.1	0.6
40	3.0	2.1	0.6
60	5.4	3.0	0.6
80	7.8	3.9	0.8
100	10.7	5.3	1.8

[1] % of oven-dried bark meal

Table 3 Total reducing sugar content in water extractives from the bark

A. mangium

Water extraction temperature (°C)	Total reducing sugars (%)[1]
60	0.5
80	0.5
100	0.8

A. auriculiformis

Water extraction temperature (°C)	Total reducing sugars (%)[1]
60	0.4
80	0.4
100	0.6

R. apiculata

Water extraction temperature (°C)	Total reducing sugars (%)[1]
60	0.4
80	0.5
100	0.6

L. leptolepis

Water extraction temperature (°C)	Total reducing sugars (%)[1]
60	0.3
80	0.3
100	0.6

[1] % of oven-dried bark meal

conditions. Water extraction at 80 °C from the bark of *A. mangium* and *L. leptolepis* is suitable for extraction of polyphenolics with the minimum possible presence of sugar components.

REFERENCES

Aoyama M, Kubota M & Takahashi H. 1983. Effects of coniferous bark extracts on plant growth. *Mokuzai Gakkaishi* 29: 930–934.

Cadahia E, Conde E, Simon BF & Garcia VMC. 1997. Tannin composition of *Eucalyptus camaldulensis, E. globules* and *E. rudis*. *Holzforschung* 51: 125–129.

Dubois M, Gilles KA, Hamilton JK, Rebers PA & Smith F 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350–356.

Ge JJ & Sakai K. 1993. Compressive properties and biodegradabilities of polyurethane foams derived from condensed tannin. *Mokuzai Gakkaishi* 39: 801–806.

Hashida K, Ohara S. & Makino R. 2006. Improvement of formaldehyde-scavenging ability of condensed tannins by ammonia treatment. *Holzforschung* 60: 178–183.

Higaki M, Kamimura N, Suzuki T & Iijima T. 1990. The properties and chemical components of mangrove: chemical components and analytical method for tannins in mangrove barks. *Mokuzai Gakkaishi* 36: 738–746.

Julkunen-Tiitto R. 1985. Phenolic constituents in the leaves of northern willows: method for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry* 33: 213–217.

Nelson N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry* 153: 375–380.

Makino R & Ohara S. 2001. Efficient extraction method of bark tannins. P. 379 in *Abstracts of the 51st Annual Meeting of the Japan Wood Research Society*.

Ohara S, Suzuki K & Ohira T. 1994. Condensed tannins from *Acacia mearnsii* and their biological activities. *Mokuzai Gakkaishi* 40: 1363–1374.

Ohara S, Fujii T & Akitsuki K. 2004a. Formation of stable charcoal layer coating the wooden material surface by the application of tannin-charcoal suspensions. *Bulletin of FFPRI* 3: 1–6.

Ohara S, Hashida K, Makino R & Yusuf S. 2004b. Tannin and related polyphenolic components from tropical tree species. Pp. 121–123 in Ona T. (Ed.) *Improvement of Forest Resources for Recyclable Forest Products*. Springer, Tokyo.

Samejima M & Yoshimoto T. 1981. General aspects of phenolic extractives from coniferous barks. *Mokuzai Gakkaishi* 27: 491–497.

Samejima M & Yoshimoto T. 1982. Accumulation and structural changes of flavanols in the barks of *Cryptomeria japonica* D. Don and *Pinus densiflora* Sieb. and Zucc. *Bulletin of the Tokyo University Forests* 72: 17–29.

Somogyi M. 1952. Notes on sugar determination. *Journal of Biological Chemistry* 195: 19–23.

Yazaki Y. 1997. *Acacia storyi*: a potential tannin-producing species. *Australian Forestry* 60: 24–28.

Yazaki Y & Collins PJ. 1994. Wood adhesives from *Pinus radiata* barks of some pine and spruce species. *Holz als Roh-und Werkstoff* 52: 185–190.