Full Length Research Paper

Molecular weights and tanning properties of tannin fractions from the *Acacia mangium* bark

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The bark of *Acacia mangium* was smashed and extracted by acetone solution, and the extract was then degreased by petroleum ether. The degreased solution was extracted by diethyl ether and ethyl acetate successively. In this way, the tannin of *A. mangium* was divided as diethyl ether fraction, ethyl acetate fraction, and water fraction, respectively. The molecular weight of these tannins was measured by gel permeation chromatography (GPC), and the particle sizes of tannin were examined by Zetasizer ZS instrument. The results showed that the ether fraction consisted of the smallest size molecule with average molecular weight of 415 Da, possessing weaker tanning ability but fast penetration rate. The molecular weight of ethyl acetate fraction was 1788 Da, and it showed a significant tanning ability. For the water fraction, the molecular weight was 2808 Da with better tanning ability, and the biggest particle size was shown in the tannin. The thermal stability of the hide powder and cowhide tanned by these tannins was as followed: Water fraction > ethyl acetate fraction > diethyl ether fraction; the penetration was: diethyl ether fraction > ethyl acetate fraction > water fraction. These results could provide a valuable reference for the use of the *A. mangium* tannin.

Key words: *Acacia mangium*, extraction, molecular weights, tanning ability tannage.

INTRODUCTION

Acacia mangium belongs to a Mimosaceae family. It is an evergreen arbor, originally planted in the Queensland Australia, southwestern New Guinea, and eastern Indonesia (Alamsyah et al., 2007). Nowadays *A. mangium* has already been widely planted in Asia as one of the most popular fast-growing trees (Tsai, 1988). As the other kind of the condensed type (Pasch et al., 2001), *A. manguim* tannin (AMT) is a condensed tannin consisting of profisetinidin, prorobintinidin, and prodelphinidin units connected through carbon-carbon bonds (Zhang et al., 2010), which contribute to the formation of oligomers and polymers with molecular weights ranging from 500 to 3000 Da. Yeoh Beng Hoong

used MALDI- TOF and CP-MAS 13CNMR to study the structure of AMT, and, for the first time, suggested that the AMT consists of 'angular' and 'twice-angular' polymer structure with more than 7 flavonoid units (Figure 1).

The tanning ability and colloid chemical properties of AMT have been well investigated (Teng et al., 2010). However, there are almost no studies on the relation between molecular weight and tanning ability of these tannins. For a better understanding of the correlation between tanning ability of tannins and their molecular weight, some different polarity organic solvents were used to extract tannin fractions having different molecular weight and polarity, providing a valuable evidence for

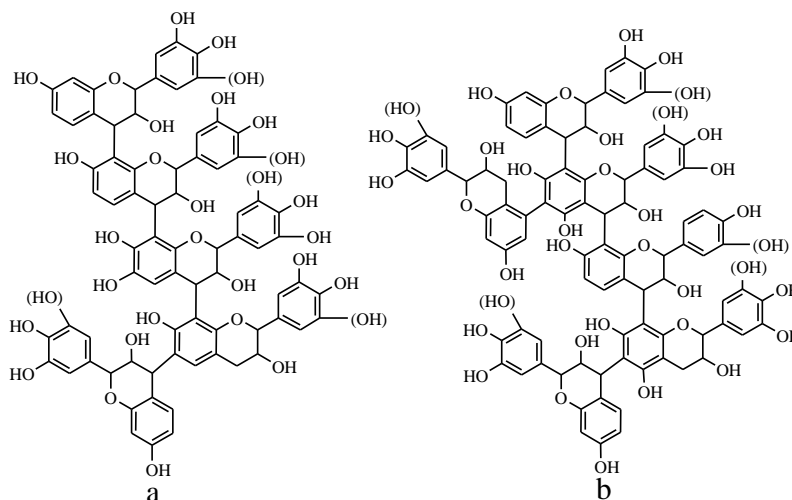*Corresponding author. E-mail: wuyong.chen@163.com. Tel/Fax: + (86)28 8540 4462.

Figure 1. Angular' (a) and 'Twice Angular' (b) Structure of *A. mangium* tannin (Hoong et al., 2010).

preparation and application of AMT in the future.

MATERIALS AND METHODS

A. mangium bark (5 years of its tree age) with 36.75% (Liang et al., 2009) tannin content was collected from Baise Tree Farm in Guangxi, China. The hide powder was purchased from Nanjin Forestry and Chemistry Research Institute. The pickle hide was collected from Zhengda Tannery Chengdu. In addition, other reagents were research grade.

Preparation of tannins

The bark was air-dried and smashed into particles of 1.0 to 2.0 mm for extraction. 3 L Acetone/water solution (7:3, v/v) was used to extract 1 kg bark at 20°C. Acetone/water extract was changed every 48 h and then was stored in dark place. Again, the acetone water extraction was degreased by petroleum ether, and then extracted by diethyl ether and ethyl acetate successively. After pressure distillation was reduced and freezing drying, 223.18 g dry extractions were obtained including 145.20 g water fraction (65% for total extraction), 74.33 g ethyl acetate fraction (33% for total extraction), and 3.56 g diethyl ether fraction (2% for total extraction). The detailed operations are shown in Figure 2.

Molecular weight distribution and particle size of tannins

One milligram of the tannin was dissolved in 1 ml tetrahydrofuran, and was subjected to gel permeation chromatography (GPC) analysis. A 150-C ALC/GPC instrument (Waters) equipped with a differential refractive index detector, and a combination of μ-Styrage GPC column (10, 50, 100 and 1000 nm) was used to measure the molecular weight of the tannins. Tetrahydrofuran (THF) as the mobile phase was pumped into the column at the flow-rate of 1.0 ml/min, 150 bars at 20°C (Cadahfa et al., 1996). A molar mass standard curve was obtained using monodispersed polystyrene as a standard sample, and the standard curve equation was $Y = 11.451 - 0.3767X$ ($R^2 = 0.9975$), which showed a good linear correlation. The diethyl ether fraction was used as a control for

testing the accuracy of the GPC experiment for 5 times. Standard deviation of the data was less than 5.17, and relative standard deviation was below 0.90%, indicating a highly accurate GPC test (Cheng, 1993). Two gram of tannin was dissolved in acetone/water (7:3, v: v) solution, and the solution was constant to 10 ml by adding acetone water (7:3, v: v) solution. Then the mixture was filtered with a 450 nm Millipore filter and transferred into the sample cell. Particle sizes of these tannins were tested using a Zetasizer Nano ZS-series instrument (Malvern) at 25°C.

Sorptive ability and binding capacity with hide powder

1.50 g tannin was dissolved in 30 ml acetone/water solution (7:3, v: v). 5 ml of acetone water solution was dried at 120°C and weighed (M_0); another 25 ml was employed as a tanning agent. After 2.00 g of dry hide powder was soaked with 25 ml distilled water in a conical flask for 4 h and 25 ml tanning agent was added and kept in a CHZ-82 shaker incubator (Jintan Fuhua) at a rotation speed of 120 r/min (20°C) rotation for 24 h. Then the mixture was filtered with Büchner funnel and 10 ml filtrate was dried at 120°C and weighed (M_1). Meanwhile the tanned hide powder (A) was collected. Sorptive ability was calculated based on the following equation:

$$\text{Sorptive ability} = 5(M_0 - M_1)/2.00 \times 100\% \tag{1}$$

The tanned hide powder (A) was washed with 1 L distilled water every 8 h, and 1% gelatin-sodium chloride solution was used to react with filtrate. After 72 h, there was no white sediment formed, which meant no reaction between gelatin-sodium chloride solution with filtrate, the washed hide powder was transferred into a oven dried at 50°C and weighed (B), and also reserved for thermal stability test. Binding ability was counted at a basis of the following equation:

$$\text{Binding ability} = (B - 2.00)/2.00 \times 100\% \tag{2}$$

Thermal stability of tanned collagen and penetration rate in leather

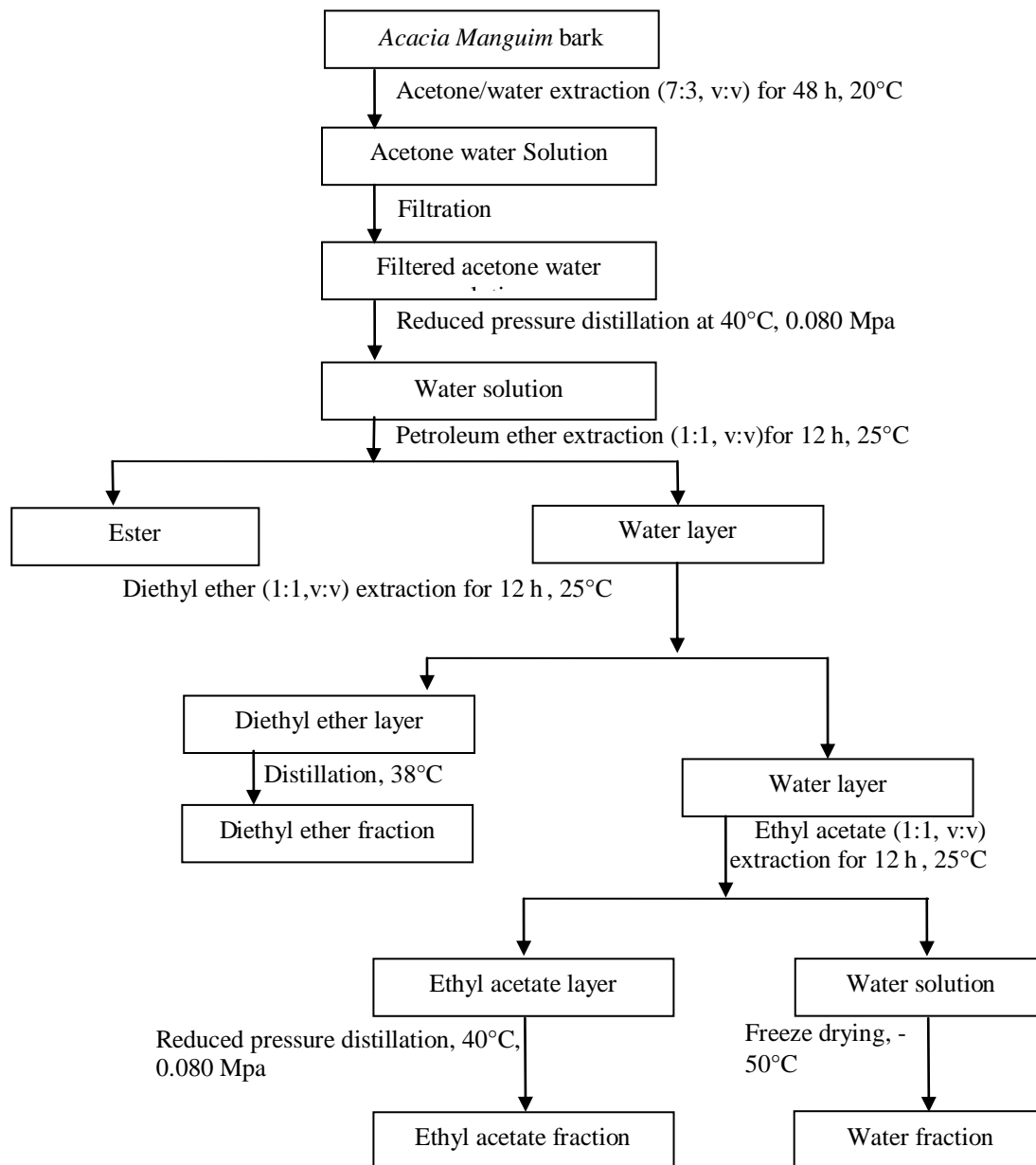The thermal properties of tanned hide powder were measured in a

Figure 2. Schematic of the extraction process.

differential scanning colorimeter DSC-2C instrument (PERKIN-ELmyer). 4 mg of tanned hide powder (B) was used. The experiments were carried out under a nitrogen atmosphere at a rate of 5°C/min, heating from 20 to 120°C, and indium was used as a control (Komanowsky, 1991). Marched skin samples (10 g average mass) were cut from pickled hide with pH 5.0. These hides were tanned by the three fractions under the same conditions. The samples were treated with 2.00 g tannins (or without tannins as blank sample) dissolved in 10 ml water acetone (7:3, v:v) solution in tanning vessel stirred at 25°C. Penetration was observed every 8 h using an eyepiece micrometer on a microscope. The penetration depth at the grain side (D_1) and flesh side (D_2) were recorded. The penetration rate was calculated as following equation:

Penetration rate = $(D_1 + D_2)$/ Leather thickness × 100%

RESULTS AND DISCUSSION

Molecular weight distributions

Table 1 shows that the $\overline{M_n}$, average polymerization degree and particle size of tannins had a quite large differences. For the diethyl ether fraction, the M_n had a relatively small molecular mass (415 Da). On the contrary, the molecular weight of the water fraction was the highest at 2808 Da. This indicated that the AMT can well be separated through some different organic solvents with different polarity. Also, there should exist

Table 1. Molecular weight and particle size of tannins.

Fraction	$\overline{\text{Mn}}$(Da)	Average polymerization degree*	Particle size (nm)
Diethyl ether fraction	415	1.4	64.11
Ethyl acetate fraction	1788	6.2	128.7
Water fraction	2808	9.7	282.9

*Date was calculated based on catechin unit (290Da).
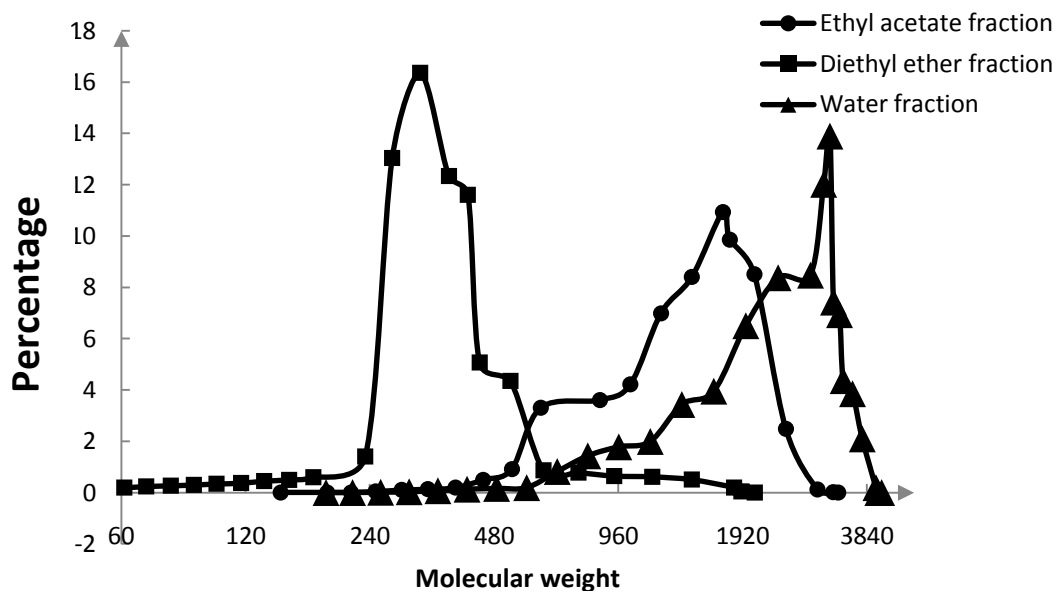
Figure 3. Molecular weight ($\overline{\text{Mn}}$) distribution of the tannins.

some relations between the molecular weight of tannin and the tanning capacity. This will be discussed thus. The Mn distribution of the tannins is shown in Figure 3. There was a narrow distribution ranging from 200 to 600 Da for the diethyl ether fraction. By contrast, for the ethyl acetate fraction and water fraction, there was a wider distribution ranging from 500 to 2500 Da and 800 to 3800 Da respectively. It is indicated that there was some overlap between these fractions.

Tanning ability

Sorptive ability and binding ability

As a first step in determining the tanning ability with different fractions, the sorptive ability and binding ability of these tannins was tested. The sorptive ability is defined as an ability of a certain amount of tannin to be bound with hide powder; the definition of binding ability is an ability of a tannin to be irreversibly combined with collagen. Results of these tests could be used for judging the tanning ability of different fractions. Table 2 shows

that the water fraction had the best properties in terms of binding and sorption. On the contrary, the diethyl ether fraction showed minimum values for both properties. This was the same as the Mn sequence. A positive correlation between the Mn and the binding ability and the sorptive ability was observed. That is to say that the higher the M n of the tannin, the stronger tanning ability it will present.
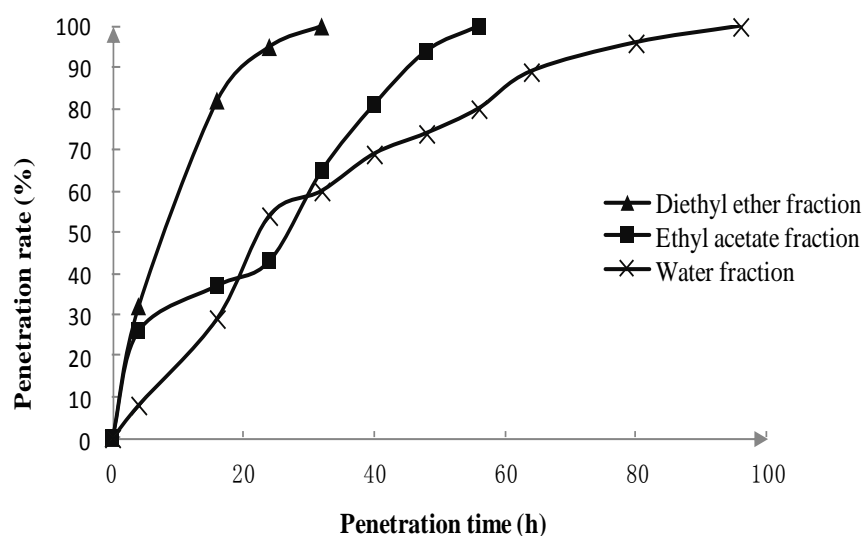
Penetration and thermal stability

Figure 4 shows the penetration time with the penetration rate of the different tannins. For the water fraction, penetration time was the longest, with up to 96 h required for its biggest particle size in the solution. Conversely, diethyl ether fraction with the smallest particle size penetrated quickly, and only 36 h was required for complete penetration. Otherwise, for ethyl acetate fraction with medium particle size, its penetration time was 56 h between diethyl ether fraction and water fraction. A positive correlation was observed between penetration rate and particle size, as determined by Mn. Thermal stability of leather can be characterized by its

Table 2. Sorptive ability and binding ability of tannins.

Ability	Diethyl ether fraction	Ethyl acetate fraction	Water fraction
Sorptive ability (%)	32	63	72
Binding ability (%)	11	47	66

Table 3. Thermal mutation temperature of tanned collagen.

Thermal (°C)	Diethyl ether fraction	Ethyl acetate fraction	Water fraction	Untreated collagen
Td	90.4	96.5	101.7	82.8
Ts	69.5	76.7	79.2	59.8

Figure 4. The penetration rate of the tannins.

shrinkage temperature (Ts) and thermal denaturating temperature (Td). White (1958) suggested that the molecular weight of vegetable tannin with tanning ability should range from 500 to 3000 Da. Vegetable tannin with molecular weight higher than 3000 Da could hardly penetrate. On the other hand, tannin with weight lower than 500 Da could not combine with collagen (White, 1954). The Td and Ts of collagen treated by water fraction was the highest, while the collagen tanned with diethyl ether fraction presented the minimum value of Td and Ts (Table 3), indicating that the Td and Ts of tanned collagen increased with the Mn (Table 1). So, it has been concluded that the tanning ability of the tannin was determined by Mn of the tannins.

different polarity and the separated fractions have different properties.

1. There is a positive correlation between the Mn and the tanning ability. In general, the higher the Mn of the tannin, the stronger tanning ability it will present.
2. The ether fraction consisted of tannin species having Mn of 415 Da with the smallest size, possessing weaker tanning ability but the fastest penetration rate.
3. The molecular weight of ethyl acetate fraction was 1788 Da, and it exhibited a significant tanning ability.
4. For water fraction, the molecular weight was 2808 Da and it had the biggest particle size, so it was judged to have the best tanning ability.

Conclusion

In summary, the AMT could well be separated to different fractions through some different organic solvents with

ACKNOWLEDGEMENT

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