Paper B18

Molecular Weights and Tanning Properties of Tannin Fractions from the Acacia Mangium Bark

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Abstract

The bark of Acacia mangium tree was smashed and extracted by acetone solution, and the extraction was then treated by petroleum ether. the solution was extracted by diethyl ether and ethyl acetate successively. So, the tanin in the bark of Acacia mangium was divided as diethyl ether fraction, ethyl acetate fraction and water fraction respectively. The GPC was used to measure the molecular weight of these fractions, and the particle sizes were examined by zetasizer ZS instrument. The results showed that the ether fraction consisted of the smallest size molecule with molecular weight of 415Da, possessing weaker tanning ability but the highest penetration rate. The Molecular weight of ethyl acetate fraction was 1788Da, owing a significant tanning ability. For water fraction, the molecular weight was 2808Da, and the biggest particle size was observed in water fraction, which had the highest tanning ability. The order of thermal stability of the collagen treated by those fractions was: water fraction> ethyl acetate fraction> diethyl ether fraction; the order of penetration rate was: diethyl ether fraction > ethyl acetate fraction> water fraction. This result could provide a valuable reference for the Acacia mangium tannin fractions.

Key words：Acacia mangium;tannin; extraction; molecular weights; tannage

1. Introduction

Acacia mangium belongs to a Mimosaceae family, evergreen arbor, originally planted in the Queensland Australia, southwestern New Guinea and eastern Indonesia [1]. Nowadays the Acacia mangium has already been widely planted in Asia as one of the most popular fast-growing trees [2-3].

Acacia manguim tannin belongs to condensed tannins and consists of profisetinidin, prorobintinidin and prodelphinidin unit connected through carbon-carbon bonds which contribute to the formation of oligomers and polymers with molecular ranging from 500 to 3000 Da[4] . Yeoh Beng Hoong used MALDI- TOF and CP-MAS ^{13}CNMR to study the structure of Acacia mangium tannin, and, for the first time, suggested that the Acacia Mangium tannin consists of ‘angular’ and ‘twice-angular’ polymer structure with more than 7 flavonoid units [5]. The tanning ability and colloid chemical properties of Acacia mangium tannin has been well investigated [6]. However, there are almost no studies on the relation between molecular weight and tanning ability of Acacia mangium tannin. For a better understanding of the correlation between tanning ability and molecular weight of Acacia Mangium tannin, organic solvent with different polarity were used to extract tannin fragments

with significant different Molecular weight, providing valuable evidences for preparation and application of Acacia mangium tannin fractions in the future.

2. Materials and method

2.1 Preparation of fractions

Acacia mangium bark with 36.75% tannin content was collected from Baise Tree Farm in Guangxi, China. The bark was air-dried and smashed into particles of 1.0–2.0 mm for extraction. 3L Acetone/water extract (3:7, v/v) was used to extract 1 kg bark at 20□. Acetone/water extract was changed every 48h and then was stored in dark place. Again,the acetone water extraction was extracted by petroleum ether, diethyl ether and ethyl acetate successively. After reduced pressure distillation and freezing drying, 223.18g extraction was obtained including 145.20g water fractions (65% for total tannins); 74.33g ethyl acetate fraction (33% for total tannins); 3.56g diethyl ether fraction (2% for total tannins). The detailed operations were shown in Fig. 1,

2.2 Molecular weight and Particle size of fractions

1 mg of the fraction was dissolved in 1mL tetrahydrofuran, and was subjected to GPC analysis. A 150-C ALC/GPC instrument (WATERS) equipped with a differential refraction detector, and a combination of μ-Styrage GPC column (10, 50, 100, 1000nm) was used to measure the molecular weight of the fractions. Tetrahydrofuran (THF) as the mobile phase was pumped into the column at the flow-rate of 1.0 mL/min, 150BARS at 20□ [7]. 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 presented well linear correlation. The diethyl ether fraction was used as a control sample 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 high accurate GPC test [8].

0.40g fraction was dissolved in acetone/water (3:7, v: v) solution and the solution was constant to 100mL by adding acetone water (3:7, v: v) solution. Then the mixture was filtered with 450nm millipore filter and transferred into sample cell. Particle sizes of these fractions were tested using a Zetasizer Nano ZS-series instrument (MALVERN) at 25□ respectively.

2.3 Tanning ability of fractions

2.3.1 Sorptive ability and binding capacity with hide powder

1.50g fraction was dissolved in 30mL acetone/water solution (1:1, v: v). 5mL of acetone water solution was dried at 120□ and weighed (M_0), another 25mL was served as tanning agent. After 2.00g dry hide powder was soaked with 25mL distilled water in a conical flask for 4h, 25mL acetone water solution was added and then kept in a water bathchader at a rotation speed of 120r/min (20□) rotation for 24h. Then the mixture was filtered with buchner funnel and 5mL filtrate was dried at 120□ and weighed (M_1), meanwhile the tanned hide powder (A) was collected. Sorptive ability was calculated based on the equation: Sorptive ability = $10(M_0-M_1)/2.00g\times100\%$.

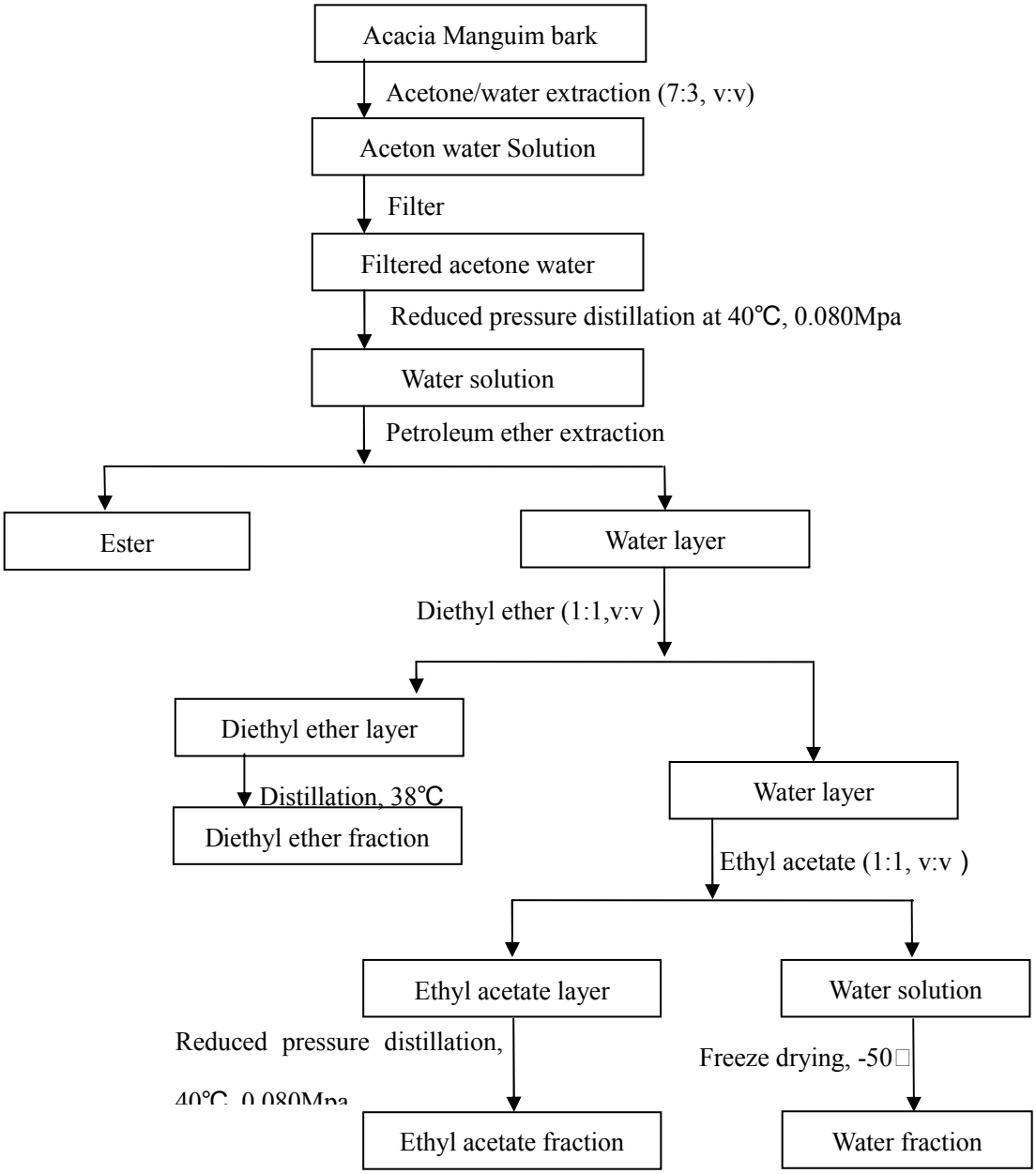

Fig 1 Schematic of the extracting process

The tanned hide powder (A) was washed with 1L distilled water every 8h, and 1% gelatin-sodium chloride solution was used to react with filtrate. When 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 and weighed (B), and the dry hide powder (C) was also reserved. Binding ability was counted at a basis of the following equation: Binding ability = (B-2.00g)/2.00g×100%.

2.3.2 Thermal stability of tanned collagen and penetration rate in leather

The thermal properties of tanned hide powder were measured in a differential scanning colorimeter DSC-2C instrument (PERKIN-ELMER). 4 mg of tanned hide powder (C) was

used. The experiments were carried out under nitrogen atmosphere by using a heating rate of 5□/min, heating from 20 to 120□, and indium was used as a control [9].

Marched skin samples (10 g average mass) were cut from pickled hide with pH 5.0. Then these hides were tanned by the three fractions under identical conditions. The samples were treated with 20% (w: w) fractions (or without fraction as blank sample) dissolved in water acetone (7:3, v:v) solution in tanning vessel stirred at 25□. Penetration rate of fractions in hide was observed every 8h using 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%,

3 Results and discussion

3.1 Molecular weight distributions

The order of n and particle size of fractions was: water fraction > ethyl acetate fraction > diethyl ether fraction (Table 1). The average polymerization degree of the three fractions was calculated based on the molecular weight of catechin unit (290Da). The results showed that the average polymerization degree of water fraction was the highest and diethyl ether fraction was lowest. The n distribution of fractions was shown in Fig. 2.

Table 1 molecular weight and particle size of fractions

	n(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

*average polymerization degree was calculated with catechin unit

White suggested that the molecular weight of vegetable tannin with tanning ability should ranges from 500 to 3000Da. For vegetable tannin agent with molecular weight higher than 3000Da, it is hard to penetrate; for tannin molecular with molecular lower than 500Da, it is hard to combine with collagen [10]. n of diethyl ether fraction was 415Da, most of which was relatively small molecule with n lower than 500, therefore this fraction should be considered as a poor tannin with little tanning ability; n of ethyl acetate fraction was 1788Da, and distribution of this fraction ranging from 500 to 3000Da, which should possess good tanning ability; but for water fraction with n of 2808Da (Fig. 2), penetration could hardly be achieved. According to the experimental results of Yeoh Beng Hoong, the average polymerization degree of Acacia Mangium tannin extracted by hot water was about 7; however, the Mn of water fraction and diethyl ether fraction was obviously deviated, proving that the fractions with different polymerization degree can be obtained by organic solvent extraction with different polarity.

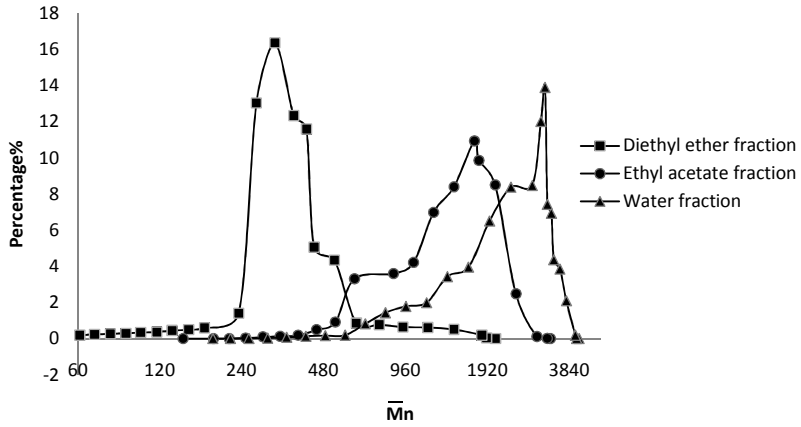

Fig 2 Molecular weight (,Mn) distribution of three fractions

3.2 Tanning ability of fractions

3.2.1 Sorptive ability and binding ability

The sorptive ability is defined as the ability of a certain amount of fraction to be bond by hide powder in the liquor. The definition of binding ability is the ability of fraction to be irreversibly combined with collagen. For the water fraction with highest molecular weight, it presented the best binding and sorptive ability. On the contrary, for diethyl ether fraction with the lowest Molecular weight, it showed the minimum value (Tab 2).Based on the above results, for Acacia mangium tannin, fractions with high n and polymerization degree possess more active hydroxyl groups in one molecular which can combine with collagen tightly; it is difficult for the tannin with lower n to bind with collagen owing the lack of hydroxyl groups.

Table 2 Sorptive ability and binding ability of fractions

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

3.2.2 Thermal stability and penetration rate

Through the DSC and leather shrinkage experiments, thermal denaturating temperature (Td) and shrinkage temperature (Ts) of collagen were obtained. The Ts and Td of collagen tanned by fractions were higher than the collagen which was not treated (Tab 3). The Td and Ts of water fraction was the highest of the three, while the diethyl ether fraction sample presented the minimum value. The Td and Ts increased with the increase of n of fractions, which proved that the thermal stability of hide powder is determined by n of fractions.

Table 3 Exothermal mutation temperature of collagen treated by fractions

	Diethyl ether fraction	Ethyl acetate fraction	Water fraction	Untreated collagen
Td℃	70.4	76.5	81.7	62.8
Ts □	69.5	76.7	79.2	59.8

This result also indicated the water fraction with high n provided abundant active sites for the crosslinking between collagen and itself and meanwhile enhance the thermal stability of collagen. But for the diethyl ether fraction with lower n, a lower thermal stability value was obtained because of a deficient crosslinking.

According to the diffusion equation of Brownian movement: $D=RT/6\eta\gamma\pi$ [11], the particle size is related to the diffusion coefficient. Particles with small size penetrate leather easily; on the contrary, it would be hard for particles to penetrate if the particle size was big. This theory was proved by the leather tanning test on this experiment. Penetration time of water fraction was 96h which was the longest because of its biggest particle size, leading to a hardship to pass through the interspaces between collagens. Conversely, for the fraction with the smallest particle size, the diethyl ether fraction can penetrate quickly, after 32h, the penetration of fraction was completed; For ethyl acetate fraction with medium particle size, its penetration time was 56h. The penetration rate curve was shown on Fig3.

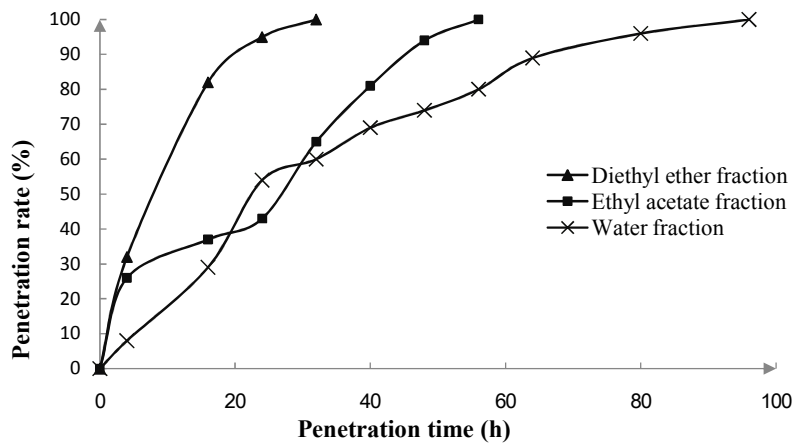

Fig 3 the penetration rate of three fractions in pickled hide

4 Conclusions

In summary, the Acacia Mangium bark was extracted by acetone solution and the solution was then treated by petroleum ether, diethyl ether and ethyl acetate successively. The molecular weight and particle size and tanning ability of fractions were tested; the tanning ability of fractions was illustrated as a function of its molecular weight. Concluded that:

For water fraction with highest molecular weight, n=2808Da. Best tanning ability and highest thermal stability of hide tanned by this fraction was shown, but biggest particle size, and longest penetration time was also observed during tanning experiment. For diethyl ether

fraction with minimum molecular weight, n=415Da. Lowest tanning ability and thermal stability were obtained, yet the smallest particle size and shortest penetration time was presented. For ethyl acetate fraction with general molecular weight, n=1788, particle size, tanning ability and penetration rate fall in between the other fractions. This result could provide valuable evidences for preparation and application of Acacia mangium tannin fractions.

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